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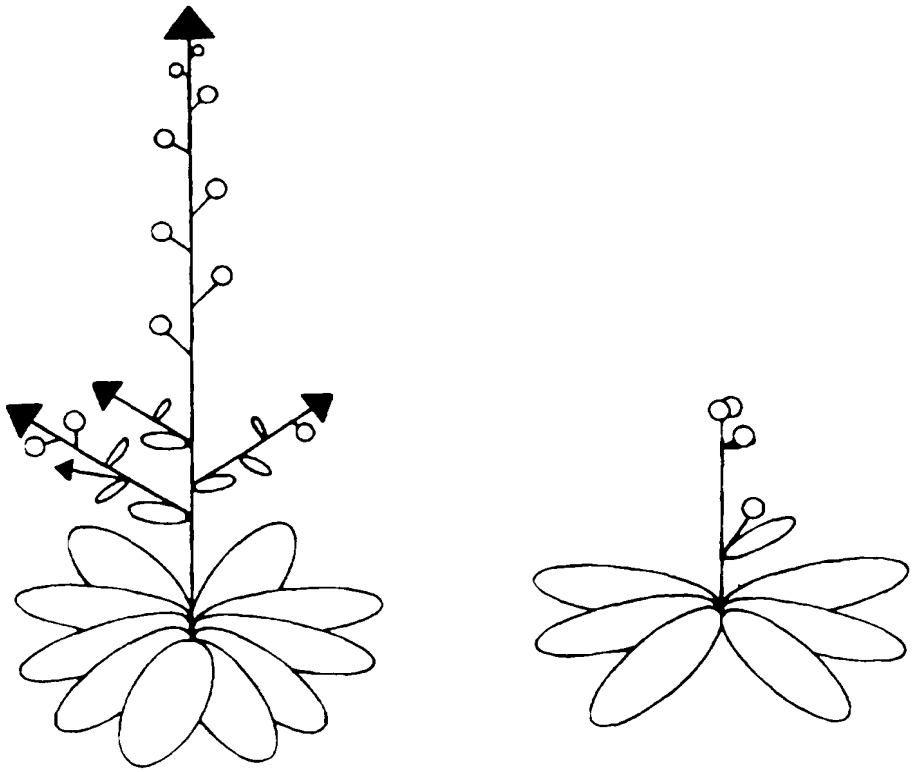
(57) Abstract

The *cen* gene of *Antirrhinum* has been cloned, also homologues from *Arabidopsis* (*tfl1*) and rice. Flowering characteristics of transgenic plants, especially switching of apical meristem to a floral fate and the timing of flowering, may be manipulated by regulating gene expression. The promoter of the *cen* gene may be used to drive tissue-specific expression, specifically in the apical meristem of plants.



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FLOWERING GENES

The present invention relates to genetic control of flowering and is based on the cloning of the *cen* gene of *Antirrhinum* and the *tfl1* gene of *Arabidopsis*.

There are three main types of meristem involved in ariel plant development; vegetative, inflorescence and floral. The apical meristem in many species, such as *Antirrhinum majus*, first undergoes a vegetative phase whereby cells set aside from the apex become leaf primordia with an axillary vegetative meristem (Coen, 1991). Upon floral induction, the apical meristem is converted to an inflorescence meristem. The traits commonly associated with the inflorescence are the modification of leaf organs and a change in internode length. The inflorescence of *Antirrhinum* is a raceme or spike, with the apical meristem growing indeterminately. Floral meristem arise in the axils of modified leaves and are determinate, producing four whorls or rings of floral organ primordia. Thus the apical meristem goes through two distinct identities, vegetative and then inflorescence. In species which produce terminal flowers, the apical meristem is determinate and eventually adopts a third identity, that of a floral meristem. A key developmental question has been to understand how the identity of the apical meristem is controlled.

The *centroradialis (cen)* mutant of *Antirrhinum* was first described in Gatersleben, Germany (Kuckuck

and Schick, 1930; Stubbe, 1966). The *cen* mutant produces a number of axillary flowers before the apical meristem is converted to a floral meristem. Thus in *cen* plants, the apical meristem goes through three distinct identities; vegetative, inflorescence and then floral. The wild-type role of *cen* is therefore to prevent the apical meristem from switching to a floral fate.

Cen mutants of *Antirrhinum* may differ from wild type in several respects. Mutants produce a terminal flower, converting the inflorescence from indeterminate to determinate. Consequently, the architecture is changed to a shorter, more bushy plant, as shoots cannot grow indefinitely. About 10 axillary flowers are made below the terminal flower. The terminal floral meristem is developmentally more advanced than the axillary flowers below it. Unlike axillary flowers, organ numbers and their arrangement (phyllotaxy) are very variable in terminal flowers. The terminal flower is usually radially symmetrical, with all petals resembling the ventral (lowest) petal of axillary flowers.

A similar mutant to *cen*, *terminal flower1 (tfl1)*, has been described in *Arabidopsis* (Shannon and Meeks-Wagner, 1991; Alvarez *et al.*, 1992). In addition to affecting meristem identity, *tfl1* mutations also result in early flowering. Therefore, the normal role of the *tfl1* gene is to inhibit flowering as well as

preventing the apical meristem from switching to a floral fate.

In *Arabidopsis*, *tfl1* mutants have two key features distinguishing from wild type: bolting early and the apical meristem eventually acquiring floral identity, leading to the production of a terminal flower (Figure 1). Typically, about half the normal number of rosette leaves are produced before bolting and about 1-5 peripheral flowers are made before the inflorescence apical meristem finally acquires floral identity. The structure of the terminal flower is often different to the wild-type. Wild-type flowers consist of 4 whorls of organs; 4 sepals outermost, 4 petals, 6 stamens and a central whorl of 2 unlimited carpels. In the terminal flower of *tfl1* mutants in *Arabidopsis*, numbers of organs often vary and they may arise in a spiral, unlike the whorled arrangement of wild-type. Mosaic organs, composed of two types of floral organ, can also be found. All of these phenotypic effects, except for a marked change in flowering time, are also seen in *cen* mutants of *Antirrhinum*.

Both these genes therefore play key roles in apical meristem identity.

To delineate the action of *cen* and the molecular pathway by which it acts, a transposon-mutagenesis programme was set up to isolate the gene. In 1992, three new alleles of *cen* (*cen*-663, *cen*-665 and *cen*-

666) were successfully isolated and a transposon linked to the *cen* phenotype in one allele was identified. Early in 1994, the flanking DNA of this transposon insertion was used to reveal that the *cen* locus had been cloned, allowing isolation of the *cen* cDNA and characterisation of its expression. CEN has similarly to a class of animal lipid-binding proteins and is expressed in the shoot apex.

The present invention is based on cloning of the *cen* gene from *Antirrhinum* and a homologue from *Arabidopsis*, *tfl1*. See also Bradley et al., *Nature* 1996, Vol. 379, 791-797 (*cen*) and Bradley, Carpenter and Coen, "Conserved control of inflorescence architecture in *Arabidopsis* and *Antirrhinum*", submitted.

According to an aspect of the present invention there is provided a nucleic acid isolate comprising a nucleotide sequence encoding a polypeptide with *cen*, *tfl1* or indeterminacy function. Those skilled in the art will appreciate that the terms "*cen* function", "*tfl1* function" and "indeterminacy function" refer to the ability to influence the timing of flowering and/or the prevention of meristems switching to a floral fate phenotypically like the respective *cen* or *tfl1* gene of *Antirrhinum* or *Arabidopsis*. "Indeterminacy function" refers to ability to keep the meristem growth indeterminantly. Certain embodiments of the present invention may have ability to

complement a *cen* or *tfl1* mutation in *Antirrhinum* or *Arabidopsis*.

Nucleic acid according to various aspects of the present invention may have the sequence of a *cen* or *tfl1* gene or be a mutant, variant, derivative or allele of the sequence provided. Preferred mutants, variants, derivatives and alleles are those which encode a product (nucleic acid molecule or polypeptide) which retains a functional characteristic of the product encoded by the wild-type gene, especially, as for *cen*, the ability to inhibit apical meristem from switching to a floral fate and/or, as for *tfl1*, the additional ability to inhibit/delay flowering. Other preferred mutants, variants, derivatives and alleles encode a product which promote flowering compared to wild-type or a gene with the sequence provided and/or promote switching of apical meristems to a floral fate. Changes to a sequence, to produce a mutant, variant or derivative, may be by one or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid, which may lead to the addition, insertion, deletion or substitution of one or more amino acids in an encoded polypeptide product. Of course, changes to the nucleic acid which make no difference to the encoded amino acid sequence are included.

In a preferred embodiment of the present invention a nucleic acid molecule comprises a

nucleotide sequence which encodes an amino acid sequence shown in Figure 4(a). The nucleotide sequence may comprise an encoding sequence shown in Figure 4(a) or may be a mutant, variant, derivative or allele thereof encoding the same amino acid sequence.

In a further embodiment, a preferred nucleic acid molecule according to the present invention comprises a nucleotide sequence encoding an amino acid sequence shown in Figure 6(a) or may be a mutant, variant, derivative or allele thereof encoding the same amino acid sequence.

Sequences comprising changes to or differences from the sequences shown in the figures may also be employed in the present invention, as discussed herein.

The present invention also provides a vector which comprises nucleic acid with any of the provided sequences, preferably a vector from which a product polypeptide or nucleic acid molecule encoded by the nucleic acid sequence can be expressed. The vector is preferably suitable for transformation into a plant cell. The invention further encompasses a host cell transformed with such a vector, especially a plant cell. Thus, a host cell, such as a plant cell, comprising nucleic acid according to the present invention is provided. Within the cell, the nucleic acid may be incorporated within the chromosome. There may be more than one heterologous nucleotide sequence

per haploid genome. This, for example, enables increased expression of the gene product compared with endogenous levels, as discussed below.

A vector comprising nucleic acid according to the present invention need not include a promoter, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome.

Nucleic acid molecules and vectors according to the present invention may be provided isolated and/or from their natural environment, in substantially pure or homogeneous form, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the sequence encoding a polypeptide with the required function. Nucleic acid according to the present invention may comprise cDNA, RNA, genomic DNA and may be wholly or partially synthetic. The term "isolate" may encompass all these possibilities.

The present invention also encompasses the expression product of any of the nucleic acid sequences disclosed and methods of making the expression product by expression from encoding nucleic acid therefor under suitable conditions in suitable host cells. Those skilled in the art are well able to construct vectors and design protocols for expression and recovery of products of recombinant gene expression. Suitable vectors can be chosen or constructed, containing appropriate regulatory

sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press. Transformation procedures depend on the host used, but are well known. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference.

Purified protein, or a fragment, mutant or variant thereof, e.g. produced recombinantly by expression from encoding nucleic acid therefor, may be used to raise antibodies employing techniques which are standard in the art. Antibodies and polypeptides comprising antigen-binding fragments of antibodies may be used in identifying homologues from other species as discussed further below.

Methods of producing antibodies include immunising a mammal (eg mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment

thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and might be screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, 1992, Nature 357: 80-82). Antibodies may be polyclonal or monoclonal.

As an alternative or supplement to immunising a mammal, antibodies with appropriate binding specificity may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, eg using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047.

Antibodies raised to a polypeptide or peptide can be used in the identification and/or isolation of homologous polypeptides, and then the encoding genes. Thus, the present invention provides a method of identifying or isolating a polypeptide with the desired function (in accordance with embodiments disclosed herein), comprising screening candidate polypeptides with a polypeptide comprising the antigen-binding domain of an antibody (for example whole antibody or a fragment thereof) which is able to bind a CEN or Tfl1 polypeptide or fragment or variant thereof or preferably has binding specificity for such

a polypeptide, such as having the amino acid sequence shown in Figure 4 or Figure 6. Specific binding members such as antibodies and polypeptides comprising antigen binding domains of antibodies that bind and are preferably specific for such a polypeptide or mutant, variant or derivative thereof represent further aspects of the present invention, as do their use and methods which employ them.

Candidate polypeptides for screening may for instance be the products of an expression library created using nucleic acid derived from an plant of interest, or may be the product of a purification process from a natural source.

A polypeptide found to bind the antibody may be isolated and then may be subject to amino acid sequencing. Any suitable technique may be used to sequence the polypeptide either wholly or partially (for instance a fragment of the polypeptide may be sequenced). Amino acid sequence information may be used in obtaining nucleic acid encoding the polypeptide, for instance by designing one or more oligonucleotides (e.g. a degenerate pool of oligonucleotides) for use as probes or primers in hybridisation to candidate nucleic acid, or by searching computer sequence databases.

The nucleotide sequence information provided herein or any part thereof may be used in a data-base search to find homologous sequences, expression

products of which can be tested for ability to influence a flowering characteristic of a plant. By sequencing homologues, studying their expression patterns and examining the effect of altering their expression, genes carrying out a similar function are obtainable.

A further aspect of the present invention provides a method of identifying and cloning *cen* homologues from plant species other than *Antirrhinum majus* which method employs a nucleotide sequence derived from any shown in the figures. The nucleotide sequence information provided herein, or any part thereof, may be used in a data-base search to find homologous sequences, expression products of which can be tested for ability to influence a plant meristem and/or other flowering characteristic. These may have *cen* or *tfl1* function or the ability to complement a respective mutant phenotype. In a preferred embodiment the sequence employed is one shared by the *cen* and *tfl1* genes provided herein. Nucleic acid libraries may be screened using techniques well known to those skilled in the art and homologous sequences thereby identified then tested.

For instance, such a method may employ an oligonucleotide or oligonucleotides which comprises or comprise a sequence or sequences that are conserved between the sequences of Figures 4 and 6 to search for homologues. Thus, a method of obtaining nucleic acid

whose expression is able to influence a flowering characteristic of a plant is provided, comprising hybridisation of an oligonucleotide or a nucleic acid molecule comprising such an oligonucleotide to target/candidate nucleic acid. Target or candidate nucleic acid may, for example, comprise a genomic or cDNA library obtainable from an organism known to contain or suspected of containing such nucleic acid. Successful hybridisation may be identified and target/candidate nucleic acid isolated for further investigation and/or use.

Hybridisation may involve probing nucleic acid and identifying positive hybridisation under suitably stringent conditions (in accordance with known techniques) and/or use of oligonucleotides as primers in a method of nucleic acid amplification, such as PCR. For probing, preferred conditions are those which are stringent enough for there to be a simple pattern with a small number of hybridisations identified as positive which can be investigated further. It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain.

As an alternative to probing, though still employing nucleic acid hybridisation, oligonucleotides designed to amplify DNA sequences may be used in PCR reactions or other methods involving amplification of nucleic acid, using routine procedures. See for

instance "PCR protocols; A Guide to Methods and Applications", Eds. Innis *et al*, 1990, Academic Press, New York.

Preferred amino acid sequences suitable for use in the design of probes or PCR primers are sequences conserved (completely, substantially or partly) between at least two polypeptides able to influence a flowering characteristic, particularly the switching of apical meristem to a floral fate, e.g. with the amino acid sequences of Figures 4 and 6 herein.

On the basis of amino acid sequence information oligonucleotide probes or primers may be designed, taking into account the degeneracy of the genetic code, and, where appropriate, codon usage of the organism from the candidate nucleic acid is derived.

Preferably an oligonucleotide in accordance with the invention, e.g. for use in nucleic acid amplification, has about 10 or fewer codons (e.g. 6, 7 or 8), i.e. is about 30 or fewer nucleotides in length (e.g. 18, 21 or 24).

Assessment of whether or not such a PCR product corresponds to resistance genes may be conducted in various ways. A PCR band from such a reaction might contain a complex mix of products. Individual products may be cloned and each one individually screened. It may be analysed by transformation to assess function on introduction into a plant of interest.

The present invention also extends to nucleic acid encoding a *cen* or *tfl1* homologue obtained using a nucleotide sequence derived from the sequence information (amino acid and/or nucleotide) presented in the figures.

Thus, included within the scope of the present invention are nucleic acid molecules which encode amino acid sequences which are homologues of *cen* of *Antirrhinum* or *tfl1* of *Arabidopsis*. Homology may be at the nucleotide sequence and/or amino acid sequence level. Preferably, the nucleic acid or amino acid sequence of a homologue, or a mutant, allele or variant (see above) shares homology with the sequence of or encoded by the nucleotide sequence of Figure 4 or Figure 6, preferably at least about 50%, or at least about 60%, or at least about 70%, or at least about 75%, or at least about 80% homology, most preferably at least about 90% homology, and the encoded product shares a phenotype with the cen and/or *tfl1* gene, preferably the ability to influence switching of apical meristem to a floral fate and/or influence timing of flowering. The influence may promote or delay such switching and/or flowering compared with wild-type. "Homology" may be understood to refer to similarity, in functional terms, in an amino acid sequence, as is standard in the art. Thus, for example, a % similarity figure will include amino acid differences that have little or no functional

significance, such as leucine to isoleucine. Otherwise, homology may be taken to refer to identity.

For example, gene homologues from economically important monocotyledonous crop plants such as rice and maize may be identified. Although genes encoding the same protein in monocotyledonous and dicotyledonous plants show relatively little homology at the nucleotide level, amino acid sequences are conserved.

In certain embodiments, an allele, variant, derivative, mutant or homologue of the specific sequence may show little overall homology, say about 20%, or about 25%, or about 30%, or about 35%, or about 40% or about 45%, with the specific sequence. However, in functionally significant domains or regions the amino acid homology may be much higher. Comparison of the amino acid sequences of the polypeptides reveals domains and regions with functional significance, i.e. a role in influencing a flowering characteristic of a plant, such as switching of apical meristem and/or timing of flowering. Deletion mutagenesis, for example, may be used to test the function of a region of the polypeptide and its role in or necessity for influence of a flowering characteristic such as timing.

Also according to the invention there is provided a plant cell having incorporated into its genome a sequence of nucleotides as provided by the present

invention, under operative control of a promoter for control of expression of the encoded polypeptide. A further aspect of the present invention provides a method of making such a plant cell involving introduction of a vector comprising the sequence of nucleotides into a plant cell and causing or allowing recombination between the vector and the plant cell genome to introduce the sequence of nucleotides into the genome.

The present invention further encompasses a plant comprising a plant cell comprising nucleic acid according to the present invention e.g. as a result of introduction of the nucleic acid into the cell or an ancestor thereof, and selfed or hybrid progeny and any descendent of such a plant, also any part or propagule of such a plant, progeny or descendant, including seed.

In certain embodiments, a plant according to the invention may be one which does not breed true. Stability, i.e. the ability to breed true, is one of the requirements of the UPOV Convention for a plant to be subject to Plant Variety Rights. Accordingly, a plant that does not breed true is not a plant variety.

The invention further provides a method of influencing the apical meristem switching and/or other flowering characteristics of a plant comprising expression of a heterologous *cen* or *tfl1* gene sequence (or mutant, allele, derivative or homologue thereof,

as discussed) within cells of the plant. The term "heterologous" indicates that the gene/sequence of nucleotides in question have been introduced into said cells of the plant or an ancestor thereof, using genetic engineering, i.e. by human intervention, for instance using appropriate transformation techniques. The gene may be on an extra-genomic vector or incorporated, preferably stably, into the genome. The heterologous gene may replace an endogenous equivalent gene, ie one which normally performs the same or a similar function or the inserted sequence may be additional to the endogenous gene. An advantage of introduction of a heterologous gene is the ability to place expression of the gene under the control of a promoter of choice, in order to be able to influence gene expression, and therefore plant phenotype, according to preference. Furthermore, mutants, variants and derivatives of the wild-type gene, eg with higher or lower activity than wild-type, may be used in place of the endogenous gene.

The principal characteristics which may be altered using the present invention are controlling the switch of meristems to a floral fate and the timing of flowering. Over-expression of the gene product of the *tfl1* gene may lead to delayed flowering; under-expression may lead to precocious flowering. Down-regulation may be achieved, for example, with "gene silencing"techniques such as anti-

sense or sense regulation, discussed further below.

This degree of control is useful to ensure synchronous flowering of male and female parent lines in hybrid production, for example. Another use is to advance or retard the flowering in accordance with the dictates of the climate so as to extend or reduce the growing season. Similarly, switching of apical meristems to a floral fate may be delayed or promoted according to the level of *cen* or *tfl1* gene product. Conversion of indeterminate growth to a terminal flower phenotype on down-regulation of *cen* or *tfl1* may allow for development of a limited number of fruits or seeds which mature, ripen and/or dry in a certain period. This may be beneficial where harvesting of immature, unripe and/or not dry fruit or grains is undersirable. For example, young and unripe canola seeds still containing chlorophyll when the cold falls in and prematurely stops the maturing and ripening process require further and costly refining of the crushed oil which is undesirably green. Grains or fruit crops over-expressing CEN/Tfl1 may be used for increasing the yield of particular crops. Changing of the architecture, in particular flowers, of ornamental plant species either from determinate to indeterminate or from indeterminate to determinate may be of commercial value.

The nucleic acid according to the invention may be placed under the control of an externally inducible

gene promoter thus placing the timing of meristem switching and/or flowering under the control of the user. The use of an inducible promoter is described below. This is advantageous in that flower production, and subsequent events such as seed set, may be timed to meet market demands, for example, in cut flowers or decorative flowering pot plants. Delaying flowering in pot plants is advantageous to lengthen the period available for transport of the product from the producer to the point of sale and lengthening of the flowering period is an obvious advantage to the purchaser.

The term "inducible" as applied to a promoter is well understood by those skilled in the art. In essence, expression under the control of an inducible promoter is "switched on" or increased in response to an applied stimulus. The nature of the stimulus varies between promoters. Some inducible promoters cause little or undetectable levels of expression (or no expression) in the absence of the appropriate stimulus. Other inducible promoters cause detectable constitutive expression in the absence of the stimulus. Whatever the level of expression is in the absence of the stimulus, expression from any inducible promoter is increased in the presence of the correct stimulus. The preferable situation is where the level of expression increases upon application of the relevant stimulus by an amount effective to alter a

phenotypic characteristic. Thus an inducible (or "switchable") promoter may be used which causes a basic level of expression in the absence of the stimulus which level is too low to bring about a desired phenotype (and may in fact be zero). Upon application of the stimulus, expression is increased (or switched on) to a level which brings about the desired phenotype.

Suitable promoters include the Cauliflower Mosaic Virus 35S (CaMV 35S) gene promoter that is expressed at a high level in virtually all plant tissues (Benfey et al, 1990a and 1990b); the maize glutathione-S-transferase isoform II (GST-II-27) gene promoter which is activated in response to application of exogenous safener (WO93/01294, ICI Ltd) (preferred in the present invention); the cauliflower meri 5 promoter that is expressed in the vegetative apical meristem as well as several well localised positions in the plant body, eg inner phloem, flower primordia, branching points in root and shoot (Medford, 1992; Medford et al, 1991) and the *Arabidopsis thaliana LEAFY* promoter that is expressed very early in flower development (Weigel et al, 1992).

An aspect of the present invention is the use of nucleic acid according to the invention in the production of a transgenic plant.

When introducing a chosen gene construct into a cell, certain considerations must be taken into

account, well known to those skilled in the art. The nucleic acid to be inserted should be assembled within a construct which contains effective regulatory elements which will drive transcription. There must be available a method of transporting the construct into the cell. Once the construct is within the cell membrane, integration into the endogenous chromosomal material either will or will not occur. Finally, as far as plants are concerned the target cell type must be such that cells can be regenerated into whole plants.

Plants transformed with the DNA segment containing the sequence may be produced by standard techniques which are already known for the genetic manipulation of plants. DNA can be transformed into plant cells using any suitable technology, such as a disarmed Ti-plasmid vector carried by Agrobacterium exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984), particle or microprojectile bombardment (US 5100792, EP-A-444882, EP-A-434616) microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green *et al.* (1987) *Plant Tissue and Cell Culture*, Academic Press), electroporation (EP 290395, WO 8706614 Gelvin Debeyser - see attached) other forms of direct DNA uptake (DE 4005152, WO 9012096, US 4684611), liposome mediated DNA uptake (e.g. Freeman *et al. Plant Cell Physiol.* 29: 1353 (1984)), or the vortexing method (e.g.

Kindle, *PNAS U.S.A.* 87: 1228 (1990d) Physical methods for the transformation of plant cells are reviewed in Oard, 1991, *Biotech. Adv.* 9: 1-11.

Agrobacterium transformation is widely used by those skilled in the art to transform dicotyledonous species. Recently, there has been substantial progress towards the routine production of stable, fertile transgenic plants in almost all economically relevant monocot plants (Toriyama, et al. (1988) *Bio/Technology* 6, 1072-1074; Zhang, *et al.* (1988) *Plant Cell Rep.* 7, 379-384; Zhang, *et al.* (1988) *Theor Appl Genet* 76, 835-840; Shimamoto, *et al.* (1989) *Nature* 338, 274-276; Datta, *et al.* (1990) *Bio/Technology* 8, 736-740; Christou, *et al.* (1991) *Bio/Technology* 9, 957-962; Peng, *et al.* (1991) International Rice Research Institute, Manila, Philippines 563-574; Cao, *et al.* (1992) *Plant Cell Rep.* 11, 585-591; Li, *et al.* (1993) *Plant Cell Rep.* 12, 250-255; Rathore, *et al.* (1993) *Plant Molecular Biology* 21, 871-884; Fromm, *et al.* (1990) *Bio/Technology* 8, 833-839; Gordon-Kamm, *et al.* (1990) *Plant Cell* 2, 603-618; D'Halluin, *et al.* (1992) *Plant Cell* 4, 1495-1505; Walters, *et al.* (1992) *Plant Molecular Biology* 18, 189-200; Koziel, *et al.* (1993) *Biotechnology* 11, 194-200; Vasil, I. K. (1994) *Plant Molecular Biology* 25, 925-937; Weeks, *et al.* (1993) *Plant Physiology* 102, 1077-1084; Somers, *et al.* (1992) *Bio/Technology* 10, 1589-1594; WO92/14828). In

particular, *Agrobacterium* mediated transformation is now emerging also as an highly efficient alternative transformation method in monocots (Hiei *et al.* (1994) *The Plant Journal* 6, 271-282).

The generation of fertile transgenic plants has been achieved in the cereals rice, maize, wheat, oat, and barley (reviewed in Shimamoto, K. (1994) *Current Opinion in Biotechnology* 5, 158-162.; Vasil, *et al.* (1992) *Bio/Technology* 10, 667-674; Vain et al., 1995, *Biotechnology Advances* 13 (4): 653-671; Vasil, 1996, *Nature Biotechnology* 14 page 702).

Microprojectile bombardment, electroporation and direct DNA uptake are preferred where Agrobacterium is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, eg bombardment with Agrobacterium coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with Agrobacterium (EP-A-486233).

Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues and organs of the plant. Available techniques are reviewd in Vasil et al., *Cell Culture and Somatic Cel Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications*, Academic Press,

1984, and Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989.

The particular choice of a transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for plant regeneration.

In the present invention, over-expression may be achieved by introduction of the nucleotide sequence in a sense orientation. Thus, the present invention provides a method of influencing a flowering characteristic, e.g. meristem switching, of a plant, the method comprising causing or allowing expression of the product (polypeptide or nucleic acid) encoded by the nucleotide sequence of nucleic acid according to the invention from that nucleic acid within cells of the plant.

Under-expression of the gene product polypeptide may be achieved using anti-sense technology or "sense regulation". The use of anti-sense genes or partial gene sequences to down-regulate gene expression is now well-established. Double-stranded DNA is placed under the control of a promoter in a "reverse orientation"

such that transcription of the "anti-sense" strand of the DNA yields RNA which is complementary to normal mRNA transcribed from the "sense" strand of the target gene. The complementary anti-sense RNA sequence is thought then to bind with mRNA to form a duplex, inhibiting translation of the endogenous mRNA from the target gene into protein. Whether or not this is the actual mode of action is still uncertain. However, it is established fact that the technique works. See, for example, Rothstein et al, 1987 *PNAS USA*, 84: 8439-8443; Smith *et al*,(1988) *Nature* 334, 724-726; Zhang *et al*,(1992) *The Plant Cell* 4, 1575-1588, English *et al*., (1996) *The Plant Cell* 8, 179-188. Antisense technology is also reviewed in reviewed in Bourque, (1995), *Plant Science* 105, 125-149, and Flavell, (1994) *PNAS USA* 91, 3490-3496.

The complete sequence corresponding to the coding sequence in reverse orientation need not be used. For example fragments of sufficient length may be used. It is a routine matter for the person skilled in the art to screen fragments of various sizes and from various parts of the coding sequence to optimise the level of anti-sense inhibition. It may be advantageous to include the initiating methionine ATG codon, and perhaps one or more nucleotides upstream of the initiating codon. A suitable fragment may have about 14-23 nucleotides, e.g. about 15, 16 or 17.

Thus, the present invention also provides a

method of influencing a flowering characteristic, e.g. meristem switching, of a plant, the method comprising causing or allowing anti-sense transcription from nucleic acid according to the invention within cells of the plant.

When additional copies of the target gene are inserted in sense, that is the same, orientation as the target gene, a range of phenotypes is produced which includes individuals where over-expression occurs and some where under-expression of protein from the target gene occurs. When the inserted gene is only part of the endogenous gene the number of under-expressing individuals in the transgenic population increases. The mechanism by which sense regulation occurs, particularly down-regulation, is not well-understood. However, this technique is also well-reported in scientific and patent literature and is used routinely for gene control. See, for example, van der Krol *et al.*, (1990) *The Plant Cell* 2, 291-299; Napoli *et al.*, (1990) *The Plant Cell* 2, 279-289; Zhang *et al.*, (1992) *The Plant Cell* 4, 1575-1588.

Thus, the present invention also provides a method of influencing a flowering and/or meristem switching characteristic of a plant, the method comprising causing or allowing expression (at least transcription) from nucleic acid according to the invention within cells of the plant to suppress activity of a polypeptide with ability to influence a

flowering characteristic. Here the activity of the polypeptide is preferably suppressed as a result of under-expression within the plant cells.

As stated above, the expression pattern of the gene may be altered by fusing it to a foreign promoter. For example, International patent application WO93/01294 of Imperial Chemical Industries Limited describes a chemically inducible gene promoter sequence isolated from a 27 kD subunit of the maize glutathione-S-transferase, isoform II gene (GST-II-27). It has been found that when linked to an exogenous gene and introduced into a plant by transformation, the GST-II-27 promoter provides a means for the external regulation of the expression of that exogenous gene.

The GST-II-27 gene promoter has been shown to be induced by certain chemical compounds which can be applied to growing plants. The promoter is functional in both monocotyledons and dicotyledons. It can therefore be used to control gene expression in a variety of genetically modified plants, including field crops such as canola, sunflower, tobacco, sugarbeet, cotton; cereals such as wheat, barley, rice, maize, sorghum; fruit such as tomatoes, mangoes, peaches, apples, pears, strawberries, bananas, and melons; and vegetables such as carrot, lettuce, cabbage and onion. The GST-II-27 promoter is also suitable for use in a variety of tissues, including

roots, leaves, stems and reproductive tissues.

Accordingly, the present invention provides in a further aspect a gene construct comprising an inducible promoter operatively linked to a nucleotide sequence provided by the present invention. This enables control of expression of the gene. The invention also provides plants transformed with said gene construct and methods comprising introduction of such a construct into a plant cell and/or induction of expression of a construct within a plant cell, by application of a suitable stimulus, an effective exogenous inducer. The promoter may be the GST-II-27 gene promoter or any other inducible plant promoter.

Ectopic expression of sense constructs may be used to inhibit flowering and convert meristems to indeterminate growth. This is useful for crops whose yield is increased by having a more extensive vegetative phase, especially when expression is later turned off. Limited expression of *cen*, for example under *plena/agamous* promoters, may cause indeterminate stems wrapped in petals, a potentially highly ornate stem.

Anti-sense or co-suppression constructs, mutant selection or other mechanisms to affect gene activity may inhibit *cen* and homologues in different species and convert indeterminate apical meristems to flowers. This may be useful in crops where tops must be pinched-off to promote laterals and "bushy"

development, or where flower number should be limited to give bigger flowers or fruits. The cut flower industry may enjoy new varieties, while the fruit tree and paper tree industries may profit from a change in branching architecture.

As discussed, the *tfl1* gene also has the effect of delaying flowering. Thus, both sense and anti-sense constructs may be used to affect flowering time. In species which benefit from delaying flowering, such as sugar beet and lettuce, or promoting flowering, transgenics may employ *tfl1* or an appropriate homologue or mutant or derivative, as discussed.

The *cen* and *tfl1* genes may be used to modulate the expression of other genes, such as *flo* or *lfy*, whose phenotypes are complementary to *cen/tfl1*, and *vice versa*.

Both molecular and phenotypic analysis indicate a mutual antagonism between *cen/tfl1* and *flo/lfy*. The normal pattern of flowering depends on how the balance between these two antagonistic activities is established. By manipulating this balance flowering may be controlled in different ways to achieve a desirable result. The phenotype of lines expressing *cen/tfl1* may be modified by changing *flo/lfy* expression and vice versa, either genetically (e.g. by crossing selected phenotypes of plants expressing *cen/tfl1* or homologues thereof with selected phenotypes of plants expressing *flo/lfy* or homologues

thereof) or transgenetically (e.g. by using expression cassettes employing a stronger or weaker promoter to drive *cen/tfl1* as compared to *flo/lfy*). For example, plants overexpressing *cen/tfl1* with a prolonged vegetative phase may be induced to flower by activation of a *flo/lfy* construct under the control of an inducible promoter.

Preliminary analysis reveals that *cen* is restricted in its expression to the apical region lying just below the shoot meristem. The *cen* promoter may therefore be employed in directed expression of genes to the apex, using suitable nucleic acid constructs.

For example, the *cen* promoter may be used to express a suitable phytotoxin to inhibit apical meristem switching into an inflorescence and/or floral meristem thereby preventing bolting and/or flowering.

Suitable phytotoxin for this purpose may include but are not limited to ribosome inhibiting proteins (Lord et al. (1991) Seminars in Cell Biol. 2:15-22, Stirpe et al. (1992) Bio/Technology 10:405-412) such as dianthin (Legname et al. (1991) Biochem. Biophys. Acta 1090:119-122), pokeweed antiviral protein (PAP) (Chen et al. (1993) Physiol. Mol. Plant Pathol. 42:237-247), ricin A (Endo and Tsurugi (1988) J.Biol.Chem. 263:8735-8739), ribonucleases such as barnase or RNAse T1 (Mariani et al. (1990) Nature 347:737-741, Mariani et al. (1992) Nature 357:384-387)

or a diphtheria toxin A chain (Thorsness et al. (1991) Dev. Biol. 143:173-184).

Accordingly, a further aspect of the present invention provides nucleic acid isolate comprising a *cen* promoter sequence, for instance a promoter sequence shown in Figure 4, or a mutant, derivative, variant, allele or homologue thereof, especially retaining ability to promote tissue-specific expression with a tissue pattern matching or similar to *cen* tissue expression pattern. The predicted promoter lies upstream in Figure 4 of NT 4327, probably within 500 nt of the start codon. The nucleic acid may be a gene construct in which a nucleotide sequence of choice is placed under control of the promoter (using appropriate orientation, spacing and so on) for expression. Techniques for nucleic acid manipulation and plant transformation, and other procedures needed to put into practice this aspect of the present invention, are disclosed above in relation to the *cen* and *tfl1* genes, homologues, mutants and derivatives.

The present invention provides a nucleic acid isolate including or consisting essentially of a promoter, the promoter including the nucleotide sequence shown in Figure 4(b) as nucleotides 1-4417 or a mutant, allele, variant, derivative, homologue, or fragment thereof which confers on the promoter ability to promote apical meristem-specific expression in a

plant.

The promoter may include one or more fragments of the sequence shown in Figure 4(b), sufficient to promote gene expression in the required tissue-specific manner. Restriction enzyme or nucleases may be used to digest the nucleic acid, followed by an appropriate assay (for example involving transforming plants with constructs including a reporter gene such as GUS operably linked to the test sequence) to determine the minimal sequence required. A preferred embodiment of the present invention provides a nucleic acid isolate with the minimal nucleotide sequence shown in Figure 4(b) required for the tissue-specific promoter activity.

By "promoter" is meant a sequence of nucleotides from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of double-stranded DNA).

"Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter. DNA operably linked to a promoter is "under transcriptional initiation regulation" of the promoter.

The present invention extends to a promoter which has a nucleotide sequence which is allele, mutant, variant or derivative, by way of one or more of nucleotide addition, insertion, substitution and

deletion in a promoter sequence as provided herein. Systematic or random mutagenesis of nucleic acid to make an alteration to the nucleotide sequence may be performed using any technique known to those skilled in the art. One or more alterations to a promoter sequence according to the present invention may increase or decrease promoter activity.

"Promoter activity" is used to refer to ability to initiate transcription. The level of promoter activity is quantifiable for instance by assessment of the amount of mRNA produced by transcription from the promoter or by assessment of the amount of protein product produced by translation of mRNA produced by transcription from the promoter. The amount of a specific mRNA present in an expression system may be determined for example using specific oligonucleotides which are able to hybridise with the mRNA and which are labelled or may be used in a specific amplification reaction such as the polymerase chain reaction. Use of a reporter gene facilitates determination of promoter activity by reference to protein production.

In various embodiments of the present invention a promoter which has a sequence that is a fragment, mutant, allele, derivative or variant, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the sequence of the promoter shown in Figure 4(b), has at least about 60% homology

with one or both of the shown sequences, preferably at least about 70% homology, more preferably at least about 80% homology, more preferably at least about 90% homology, more preferably at least about 95% homology. The sequence in accordance with an embodiment of the invention may hybridise with one or both of the shown sequences, or the complementary sequences (since DNA is generally double-stranded).

Further provided by the present invention is a nucleic acid construct including or consisting essentially of a promoter according to the invention operably linked to a nucleotide sequence to be expressed, e.g. a coding sequence or sequence encoding desired RNA (e.g. for sense or anti-sense regulation). The gene may be heterologous, by which is meant a sequence other than that of *cen*. Generally, the sequence may be transcribed into mRNA which may be translated into a peptide or polypeptide product which may be detected and preferably quantitated following expression. A gene whose encoded product may be assayed following expression is termed a "reporter gene", i.e. a gene which "reports" on promoter activity.

Further provided as aspects of the present invention are vectors constructs and host cells containing nucleic acid including a promoter according to the invention. Host cells may be microbial or plant. Plants comprising such plant cells, whether

varieties or not, are also provided by the present invention, as is the use of the nucleic acid in the production of a transgenic plant. Methods of causing or allowing expression from the promoter in host cells, such as plant cells, which may be in plants, represent further aspects of the invention.

All documents mentioned herein are incorporated by reference.

Experimental work which lead to the making of the present invention will now be described with reference to the accompanying figures.

Figure 1: Cartoons of *tfl1* mutant and wild-type plants.

In wild-type (Figure 1a), the inflorescence grow indefinitely and flowers (circles) are generated from the periphery of indeterminate inflorescence meristems (filled arrow heads). Secondary inflorescences (coflorescences) arise in the axils of stem leaves. In *tfl1* plants (Figure 1b), inflorescences are often replaced by a single, terminal flower.

Figure 2: Genomic DNA blot.

DNA from the wild-type *Antirrhinum* progenitor line (JI.2 WT), the original Gatersleben *cen* allele (*cen*-594) and three new *cen* alleles (663, 665 and 666

identified in the F1 population arising from a cross between mutagenised JI.WT plants and *cen*-594, were digested with EcoRI, blotted and probed with the flanking region of pJAM2017 (see Figure 3). A wild-type F1 sibling generated in the mutagenesis (sib) and a wild-type revertant (Rev+) arising from the *cen*-594 allele, were treated similarly.

Figure 3: The *cen* locus.

Figure 3 (a) Map of the *cen* genomic region carrying the *cen*-663 allele. The insertions site of the transposon Tam6 is shown with EcoRI, E, and XbaI, X, sites indicated. The internal Tam6 XbaI fragment used to isolate the 6.0 kb EcoRI fragment, segregating with the *cen* phenotype of plants carrying *cen*-663, is flanked by an EcoRI site (E) that only partially cut in genomic DNA digests. This allowed the isolation of the 6.0 kb fragment from *cen*-663 and was cloned as pJAM2017. The 2 kb flanking region (an AccI,A, to EcoRI fragment) used to probe the genomic DNA of Figure 2 is shown as a thicker line below the locus. The 6.5 kb EcoRI wild-type genomic fragment was subcloned as pJAM2018. The 7 kb BamHI, B, was subcloned as pJAM2019. Sequencing of these wild-type clones revealed two regions with similarity to upstream regions of the *Antirrhinum* genes *globosa* and *FIL1*, indicated by open boxes and marked g and f, respectively.

Figure 3 (b) Structure of the *cen* gene and the insertion of the transposon-generated alleles determined by sequencing. Exons are represented by boxes, filled for coding and open for untranslated. Introns are indicated by horizontal lines. Triangles upon vertical lines indicate the transposon insertion sites of the alleles indicated. The arrow shows the direction of transcription.

Figure 4

Figure 4(a) shows the nucleotide sequence of *cen* cDNA complied from 5' and 3' RT-PCR products and comparison with the genomic sequence. The deduced amino acid sequence and the longest open reading frame is shown below.

Figure 4(b) shows the genomic sequence containing the *cen* gene. The *cen* cDNA sequence is given in lower case with the predicted amino acid sequence below. Upper case shows the 5' and 3' regions and the introns. The promoter sequence is included.

Figure 5: Similarly of *cen* to animal lipid-binding proteins.

The amino acid sequences (one letter code) for the deduced protein gene products of *cen* of *Antirrhinum* (Cen), morphine- or lipid-binding protein of rats (Pbp1) and bovine phosphatidylethanolamine-binding protein (Pbp) are shown.

Figure 6:

Figure 6(a) shows the nucleotide sequence of *tfl1* cDNA obtained from an *Arabidopsis* EST, and the predicted encoded amino acid sequence. Point mutations were detected in *tfl1* alelles as indicated, with the underlined base substituted with the base directly above. These mutations result in changes in the encoded amino acid sequence: glycine to aspartate in *tfl1-1*, glycine to serine in *tfl1-11*, glutamate to lysine in *tfl1-13* and threonine to isoleucine in *tfl1-14*.

Figure 6(b) shows the genomic sequence of the Arabidopsis clone containing the EST cDNA clone 129D7T7. The EST cDNA sequence is given in lower case with the predicted amino acid sequence below. Upper case shows the 5' and 3' regions and the introns.

Figure 7: *Arabidopsis* and rice Expressed Sequence Tags with similarity to *cen*.

The *Arabidopsis* clone (Arab) was completely sequenced and appeared to be full length, while the rice clone (Rice1946) was only sequenced at the 3' end. Data also suggested that the rice clone was a cDNA from an unprocessed transcript. Therefore, only the likely 3' coding region was translated to give the predicted peptide shown. A separate rice clone from the database, Rice2918, was also likely to be unprocessed and therefore two peptides, a and b,

similar to those of exons 2 and 3 of *cen*, were translated for comparison.

Figure 8: Plasmid constucts for ectopic expression of *cen* and *tfl1*.

The *cen* and *tfl1* open reading frames were cloned downstream of the Cauliflower 35S promoter and inserted into binary vectors (SLJ44024A) to give plasmids pJAM2075 (Figure 8(a)) and pJAM2076 (Figure 8(b)) respectively.

Materials and Methods

Plants

The original *cen* allele, *cen*-594, was obtained from Gatersleben, Germany. A derivative of stock JI.2 was used that contained a *globosa* allele. Plants of this JI. line were grown at 15°C and then used in crosses with *cen*-594 also grown at 15°C (Carpenter *et al.*, 1987). Progeny from these crosses were grown and three new *cen* alleles, *cen*-663, *cen*-665 and *cen*-666 were obtained. These F1 plants and three wild-type siblings from each family were maintained as cuttings (Carpenter and Coen, 1995).

DNA and RNA Analysis.

The methods for DNA and RNA extraction and blot analysis were as described previously (Coen *et al.*, 1986; Coen and Carpenter, 1988). The Tam6 fragment

used in screening was a 4 kb XbaI fragment which was flanked on either side by EcoRI sites (see map of Figure 3; Doyle *et al.*, unpub). The 6.0 kb EcoRI fragment identified in *cen*-663 with Tam6 was isolated by digesting genomic DNA from a homozygous *cen*-663 plant (obtained from selfing of the original F1), fractionating DNA by agarose gel electrophoresis and electroeluting a 5-7 kb size fraction, purifying this by ion-exchange chromatography using a NACS PREPAC column (Bethesda Research Laboratories, Inc.) and ligating to EcoRI digested and phosphatased lambda gt10 arms as described in the Kit protocol (Amersham cDNA rapid cloning module- lambda gt10 code RPN1713). Packaging *in vitro* (Amersham module N334L) gave a library of about 150,000 recombinants, which was screened using the Tam6 probe. One positive was isolated and purified that contained a 6.0 kb EcoRi fragment, though 3.6 and 2.4 kb bands were present in varying amounts. The 6.0 kb fragment was subcloned in to Bluescript vector KS+ (Stratagene) to give pJAM2017 and, when mapped, revealed an internal EcoRI site that gave 3.6 and 2.4 kb fragments. This suggested that the 6.0 kb band was only partially digested, as expected from the map of Tam6 and the internal XbaI probe used in screening. The region flanking Tam6 (a 2 kb AccI-EcoRI fragment) was used to screen a lambda EMBL4 library of wild-type *Antirrhinum* DNA, partially digested with Sau3A. From about 500,000 recombinants,

7 overlapping clones were isolated, with inserts of average size 15-16 kb. These clones were used to construct a map of the genomic region and to determine the approximate positions of the insertions responsible for the different alleles. Exact insertion sites were determined using PCR on genomic DNA of each allele, with oligonucleotides to *cen* in both directions, and a conserved oligo to the CACTA family of transposable elements (Doyle *et al.*, unpub.). The 6.5 kb genomic clone, pJAM2018, contained the insertion sites of all alleles but did not identify any cDNA clones when used as a probe against a cDNA library constructed from poly(A) RNA isolated from young inflorescences of wild-type *Antirrhinum* (Simon *et al.*, 1994). Therefore, a small region (about 200 bp) flanking the *cen*-663 allele was sequenced by the dideoxynucleotide method (Chen and Seeburg, 1985) using Sequenase version 2 from United States Biochemical Corporation. Oligos based on this sequence were designed in both directions, in possible Open Reading Frames, for RT-PCR on total RNA from wild-type and *cen* mutants young inflorescences. This identified a cDNA originating from the region flanking the insertion in *cen*-663 which was not expressed in each of the alleles. This partial *cen* cDNA was subcloned in to Bluescript vector KS+ as pJAM2020. Both the genomic and cDNA clones were fully sequenced and the intron-exon boundaries determined. The 5'end

of the *cen* mRNA was determined using the kit, 5'RACE system for rapid amplification of cDNA ends (GibcoBRL). The complete *cen* cDNA was constructed from the different RT-PCR products using convenient restriction enzyme sites. Database searches involved BLASTN (Altschul *et al.*, 1990) and FASTA (Pearson and Lipman, 1988).

The *Arabidopsis* clone 129D7T7 was obtained from the *Arabidopsis* Biological Resource Center at Ohio State and was originally isolated from *A.thaliana* var Columbia and partially sequenced by Newman *et al.*, at MSU-DOE, Michigan (Accession No. T44654). The rice clone S1946_1A was obtained from Sasaki *et al.*, National Institution of Agrobiological Resource Rice Genome Resource Project, Ibaraki, Japan and was isolated from *Oryza sativa* (Accession No. D40166). The partial sequence of the rice clone R2918_1A was obtained from the databases. Mapping of the Arabidopsis cloned was as described (Schmidt *et al.*, 1994).

In situ Hybridisation

The methods for digoxigenin labelling of RNA probes, tissue preparation and *in situ* hybridisation were as described (Bradley *et al.*, 1993). An internal AccI-RsaI fragment of the partial *cen* cDNA, pJAM2020, was subcloned in to Bluescript vector KS+ and used to generate antisense and sense control probes using T3

and T7 polymerase. An internal fragment of *tfl* was generated by PCR, subcloned into pGEM-T vector (Promega) to give plasmid pJAM2045, and used to generate antisense and sense probes using T7 and SP6 polymerases.

Constructs and transformation

The *cen* and *tfl1* open reading frames were isolated and each used to replace the GUS gene of plasmids SLJ4D4 and SLJ4K1 respectively (Jones et al., 1992). The *cen* and *tfl1* open reading frames, flanked by the CaMV 35S promoter and ocs or nos terminators respectively, were isolated and cloned into the binary vector SLJ44024A (Jones et al., 1992) to give pJAM2075 and pJAM2076. Transformation of *Arabidopsis* was made by vacuum infiltration (Bechtold et al., 1993) and root tranformation and regeneration (Valvekans et al., 1988).

Results

Isolation of new *cen* alleles

Early analysis of the original *cen* allele obtained from Gatersleben (*cen*-594) suggested that it was not very unstable and, therefore, that it might not be transposon-induced. Furthermore, it was in a line that might carry quite a different array of transposons from the probes available to those present in John Innes lines. Therefore, in 1990, a directed-

tagging approach was set up using transposon-active John Innes lines, grown at 15°C, crossed to the Gatersleben allele. The line chosen was a derivative of stock JI.2 and contained a new *globosa (glo)* allele, which suggested that transposons were possibly active in the *glo* region. Early mapping data suggested linkage between *cen* and *glo*, so this line may have provided a source of active transposons in the vicinity of *cen* (Stubbe, 1966). Also, because transposons tend to jump to linked sites, the frequency of insertions at *cen* could be enhanced in this line (Coen *et al.*, 1988). In 1992, from a screen of about 10,000 plants, three new alleles of *cen* were successfully isolated in the F1 generation. The production of these alleles provided a unique resourse that was instrumental in allowing *cen* to be isolated.

Description of wild type and *cen* mutants

Early development in all *cen* alleles was as wild type; the apical meristem undergoing a vegetative phase producing leaves bearing dormant or further vegetative shoots. Upon flowering the apical meristem in both wild type and *cen* mutants switched to producing modified leaves (bracts) bearing flowers in their axils. However, while wild type maintained this state, the apical meristem of *cen* plants was converted to a flower after a number of axillary flowers had been produced (Figure 1). In greenhouse or controlled

environmental conditions (16hr daylength and 20-25°C) about 5 to 20 axillary flowers were made on the main shoot of each allele, and fewer on lateral shoots. The new alleles showed variation in both the number of axillary flowers made before the terminal flower and in the morphology of the apical flower. A range of symmetries in apical flowers could be found, from radially symmetrical to a morphology closer to that of axillary flowers.

Cloning of *cen*

Genomic DNA from *cen*-663 and three wild-type F1 siblings were digested with EcoRI and probed with transposons Tam1 to Tam8. A Tam6 probe gave a 6.0 kb band that was uniquely present in *cen*-663 and linked to the *cen* phenotype. Linkage was established by probing DNA from individuals of an F2 family, from a backcross of *cen*-663 to wild type, stock JI.2. The fragment was cloned by isolating a 5-7kb fraction of EcoRI-digested genomic *cen*-663 DNA, ligating to a lambda vector and screening the resulting library with Tam6. A positive clone was isolated and its insert subcloned in to Bluescript vector KS+ to give pJAM2017. This clone was mapped and the flanking region used as a probe against DNA from different *cen* alleles and wild-type siblings (Figure 2). The expected 6.0 kb band and variable levels of a 2.5 kb band (a derivative of the 6.0 kb fragment, explained

below) were detected in *cen*-663, whereas the wild type progenitor, JI.2, gave a 6.5 kb band. The allele from Gatersleben, *cen*-594, used as the parent in the directed-tagging experiment, gave 8.1 and 2.5 kb bands. As expected, these two bands were present in all F1 *cen* alleles and their wild-type siblings. However, each *cen* mutant had lost the progenitor wild-type band of 6.5 kb. In *cen*-665, a new band of 3.4 kb was present, while *cen*-666 and neither the wild-type or any new band. The *cen*-666 allele was never obtained in a homozygous state and appeared to carry a deletion of unknown size. Proof that we had cloned part of the *cen* locus came from analysis of revertants Progeny of homozygous *cen*-594, *cen*-663 and *cen*-665 grown and selfed at 15°C gave revertant progeny with a wild-type phenotype, indicating that these alleles were each caused by a transposon insertion. The revertants in each case had a restored wild-type band of 6.5 kb and the corresponding mutant band of each allele, as expected from their heterozygous phenotypes (Figure 2).

Overlapping clones from a wild-type genomic library were isolated and used to construct a map of the *cen* region (Figure 3a). The wild-type 6.5 kb EcoRI fragment was subcloned as pJAM2018 and fully sequenced. The insertions causing the different *cen* alleles were first mapped by genomic DNA blots. Using a conserved oligonucleotide (oligo) to the CACTA end

of a family of transposons in *Antirrhinum*, in combination with oligos to the *cen* region (see below), the alleles indicated were precisely mapped (Figure 3b). The different insertions indicated that the right-hand end of the 6.5 kb EcoRI fragment was critical to *cen* function. However, when this and other regions of pJAM2018 were used to probe a cDNA library made from poly(A) RNA from wild-type *Antirrhinum* young inflorescences, no hybridising clones were detected. Since RNA blots similarly proved inconclusive, about 200 bp flanking the *cen*-663 allele was sequenced. A number of oligos, based on this sequence and possible open reading frames (ORF) in both directions, were synthesised and used in RT-PCR on total RNA from wild-type *Antirrhinum* or *cen* mutants, young inflorescences or vegetative shoots and leaves. Only oligos pointing in the same direction (left to right, 5' to 3' in the map of Figure 3) gave a PCR product and this was absent from RNA of the *cen* alleles.

The 3' PCR cDNA was cloned as pJAM2020 and the 5' end of the *cen* mRNA was determined by 5' RACE-PCR. The complete predicted cDNA and ORF were determined (Figure 4). The transcription unit consisted of 4 exons comprising about 930 bp. The ORF had the potential to encode a 181 amino acid protein of 20.3 kDa Mr. Searches against databases revealed most similarity to a family of lipid-binding proteins

present in animals (Figure 5). Regions of significant similarity extended throughout the protein and a potential nucleotide-binding region was partly conserved (CEN residues 116-132). These proteins may also complex with GTP-binding proteins, but the domains for both functions have not been clearly defined.

Using the *cen* cDNA as a probe, a genomic library of wild-type *Arabidopsis thaliana* var Columbia was probed at moderate stringency. One strongly hybridising clone was isolated and the region most similar to the probe was fully sequenced (Figure 6). Meanwhile, database searches identified an *Arabidopsis* Expressed Sequenced Tags (EST) clone 129D7T7 that had similarity to *cen*. Complete sequencing of the *Arabidopsis* clone revealed the predicted protein (Arab), to be 70% identical and about 82% similar to cen (Figure 7). The *Arabidopsis* EST sequence was identical to the genomic clone and was allowed the intron-exon structure to be determined (Figure 6). This was very similar to the *cen* gene, with identical positions for the introns. Further database searches identified two rice clones (S1946_1A and R2918_1A) whose partial sequences appeared to have introns at positions similar to *cen*. These sequences predicted a C-terminal, 60 amino acid peptide with 80% identity to the end of *cen* for Rice1946, and two predicted peptides (Rice2918a and b) that showed high similarity

to exons 2 and 3 of *cen* (Figure 7).

Identifying *tfl1* as a homologue to *cen*

The Arabidopsis clone, 129D7T7, was mapped using the closest available RFLP and YAC markers to the end of the chromosome 5. The *tfl1* mutation maps to this region. Primers based on this sequence were used in PCR to isolate the corresponding genomic region in four alleles of *tfl1* (*tfl1*-1, *tfl1*-11, *tfl1*-13 *tfl1*-14).

For sequence comparison of the different *tfl1* alleles, wild-type *Arabidopsis* (Columbia) and plants carrying *tfl1* alleles *-1*, *-11*, *-13* or *-14*, were grown on soil under long days, and genomic DNA was isolated using a miniprep method. Leaf tissue was homogenised while frozen, buffer added (50 nM EDTA, 0.1M Tris-HCL pH8, 1% SDS) and the sample thawed at 65°C for 2 min. DNA was extracted with phenol, phenol-chloroform, chloroform, and precipitated with isopropanol/Na acetate. After an ethanol wash, DNA was resuspended in TE containing RNase. Oligonucleotide primers were designed to sequences about 160 bp upstream of the ATG and 120 bp downstream of the stop codon. To avoid PCR artefacts, three separate PCRs were carried out on each DNA preparation and one PCR product from each was cloned into pGEM-T vector (Promega). Each clone of about 1.3 kb was sequenced using the ABI Prism system (Perkin-Elmer) and only base changes present in all 3

PCR products for any one allele were considered genuine.

All four alleles show mutations that would disrupt the predicted *Arabidopsis* protein, proving that this gene is *tfl1*. The changes are shown in Figure 6(a), and were single nucleotide mutations as indicated in the figure, resulting in the following amino acid changes: in *tfl1-1* - glycine to aspartate, in *tfl1-11* - glycine to serine, in *tfl1-13* - glutamate to lysine, and in *tfl1-14* - threonine to isoleucine. (The mutant sequences, both nucleotide and amino acid, each represent an aspect of the present invention.)

Expression studies of *cen* and *tfl1*

The timing and histological distribution of *cen* and *tfl1* RNA was determined by *in situ* hybridisation using digoxigenin-labelled *cen* on *tfl1* antisense RNA against wild-type tissue of *Antirrhinum* and *Arabidopsis* respectively. In wild-type, *cen* and *tfl1* are expressed in the shoot apex of young inflorescences, in the region immediately below the apical meristem.

Ectopic expression of *tfl1* and *cen* in *Arabidopsis*

To overexpress *cen* and *tfl1*, their respective open reading frames were cloned downstream of the Cauliflower 35 S promoter and inserted into binary vectors to give plasmids pJAM2075 and pJAM2076 (Figure

8) and used for transformation. One transformant was obtained with the 35S-*cen* construct and showed a delay in bolting and flowering and a conversion of flowers to leafy shoots. Six transformants were obtained with 35S-tfl and all showed a conversion of flowers to leafy shoots. They also displayed a range of flowering and bolting times and in the most severe cases, flowering was greatly delayed compared to wild type (more than double the normal number of rosette leaves). Taken together these results show that ectopic expression of *cen* or *tfl1* can delay flowering. Furthermore, the ability of the *cen* gene of *Antirrhinum* to modify flowering time in *Arabidopsis* shows that these genes can act across wide taxonomic distances.

References

Altschul, S.F. et al., (1990) J.Mol.Biol. 215; 403-410.

Alvarez, J. et al., (1992) The Plant J. 2; 103-116.

Bechtold, N. et al., (1993) R. Acad. Sci., Paris 316 1194-1199.

Bradley, D. et al., (1993) Cell 72; 85-95.

Carpenter, R. et al., (1987) Mol.Gen.Genet. 207; 82-89.

Carpenter, R. et al., (1995) Development 121; 19-26.

Chen, E.Y. et al., (1985) DNA 4; 165-170.

Coen, E.S. et al., (1991) Annu.Rev.Plant Physiol. Plant Mol. Biol. 42; 241-279.

Coen, E.S. et al., (1988) EMBO J. 7; 877-883.

Coen, E.S. et al., (1989) In Mobile DNA, Berg, D.E. and Howe, M.M. (eds), American Society for Microbiology, Washington.

Jones, J.D.G. et al., (1992) Trans. Res. 1; 285-297.

Kukuck, H. et al., (1930) indikt. Abst.-u. vererbungsl., 56; 51-83.

Pearson, W.R. et al., Proc.Natl.Acad.Sci. USA. 85; 2444-2448.

Schmidt, R. et al., (1994) Plant J. 5; 735-744.

Shannon, S. et al., (1991) The Plant Cell 3; 877-892.

Simon, R. et al., (1994) Cell 78; 99-107.

Stubbe, H. (1966) (ed). Genetik und Zytologie von *Antirrhinum* L. sect *Antirrhinum*. VEB Gustav Frischer Verlag, Jena.

Valvekans, D. et al., (1988) Proc. Natl. Acad. Sci. USA 85; 5536-5540.

CLAIMS:

1. A nucleic acid isolate having a nucleotide sequence coding for a polypeptide which includes the amino acid sequence shown in Figure 4(a).

2. Nucleic acid according to claim 1 wherein the coding sequence is the coding sequence shown in Figure 4(a).

3. Nucleic acid according to claim 1 wherein the coding sequence is a mutant, allele or variant of the coding sequence shown in Figure 4(a).

4. A nucleic acid isolate having a nucleotide sequence coding for a product which includes a sequence mutant, allele, variant or derivative of the CEN amino acid or nucleotide sequence of the species *Antirrhinum majus* shown in Figure 4(a) or a homologue from another species, by way of insertion, deletion, addition or substitution of one or more residues, or a said homologue, wherein expression of said product in a transgenic plant influences flowering characteristics of said plant.

5. Nucleic acid according to claim 4 wherein said flowering characteristics include the switching of apical meristem to a floral fate.

6. Nucleic acid according to claim 5 wherein said product has the ability to inhibit apical meristem from switching to a floral fate.

7. Nucleic acid according to claim 5 wherein said product has the ability to promote apical meristem switching to a floral fate.

8. Nucleic acid according to claim 4 or claim 5 wherein said flowering characteristics include the timing of flowering.

9. Nucleic acid according to claim 8 wherein said product has the ability to advance flowering in a plant.

10. Nucleic acid according to claim 8 wherein said product has the ability to delay flowering in a plant.

11. Nucleic acid according to claim 5 wherein said homologue is an *Arabidopsis* homologue.

12. Nucleic acid according to claim 11 wherein said homologue encodes the amino acid sequence shown in Figure 6(a).

13. Nucleic acid according to claim 12 having the coding sequence shown in Figure 6(a).

14. Nucleic acid according to claim 13 wherein the coding sequence is a mutant, allele, variant or derivative of the coding sequence of Figure 6(a).

15. A nucleic acid isolate comprising a nucleotide sequence coding for a product comprising a sequence mutant, allele, variant or derivative of the product encoded by the nucleic acid of claim 13, by way of insertion, deletion, addition or substitution of one or more residues, which mutant, allele, variant or derivative has at least about 70% homology with the amino acid sequence or nucleotide sequence of Figure 6(a) and ability to influence a flowering characteristic of a plant.

16. Nucleic acid according to any of claims 1 to 15 further including a regulatory sequence for expression of said coding sequence.

17. Nucleic acid according to claim 16 wherein the regulatory sequence includes an inducible promoter.

18. A nucleic acid isolate having a nucleotide sequence complementary to a coding sequence of any of claims 1 to 15, or a fragment of a said coding sequence suitable for use in anti-sense regulation of expression.

19. Nucleic acid according to claim 18 wherein said nucleotide sequence complementary to a said coding sequence or a fragment thereof is under control of a regulatory sequence for anti-sense transcription.

20. Nucleic acid according to claim 19 wherein the regulatory sequence includes an inducible promoter.

21. A nucleic acid vector suitable for transformation of a plant cell and including nucleic acid according to any preceding claim.

22. A host cell containing heterologous nucleic acid according to any preceding claim.

23. A host cell according to claim 22 which is microbial.

24. A host cell according to claim 23 which is a plant cell.

25. A plant cell according to claim 24 having said heterologous nucleic acid within its genome.

26. A plant cell according to claim 25 having more than one said nucleotide sequence per haploid genome.

27. A plant comprising a plant cell according to any

of claims 24 to 26.

28. A plant according to claim 27 which does not breed true.

29. Selfed or hybrid progeny or a descendant of a plant according to claim 27 or claim 28, or any part or propagule of such a plant, progeny or descendant, such as seed.

30. A method of influencing a flowering characteristic of a plant, the method comprising causing or allowing expression of a product encoded by heterologous nucleic acid according to any of claims 1 to 17 within cells of the plant.

31. A method of influencing a flowering characteristic of a plant, the method comprising causing or allowing transcription from heterologous nucleic acid according to any of claims 1 to 17 within cells of the plant.

32. A method of influencing a flowering characteristic of a plant, the method comprising causing or allowing anti-sense transcription from nucleic acid according to any of claims 18 to 20 within cells of the plant.

33. Use of nucleic acid according to any of claims 1 to 17 in the production of a transgenic plant.

34. Use of nucleic acid according to any of claims 18 to 20 in the production of a transgenic plant.

35. A nucleic acid isolate including a promoter, the promoter including the nucleotide sequence shown in Figure 4(b) as nucleotides 1-4417 or a mutant, allele, variant, derivative, homologue, or fragment thereof which confers on the promoter ability to promote apical meristem-specific expression in a plant.

Fig.1 (a).

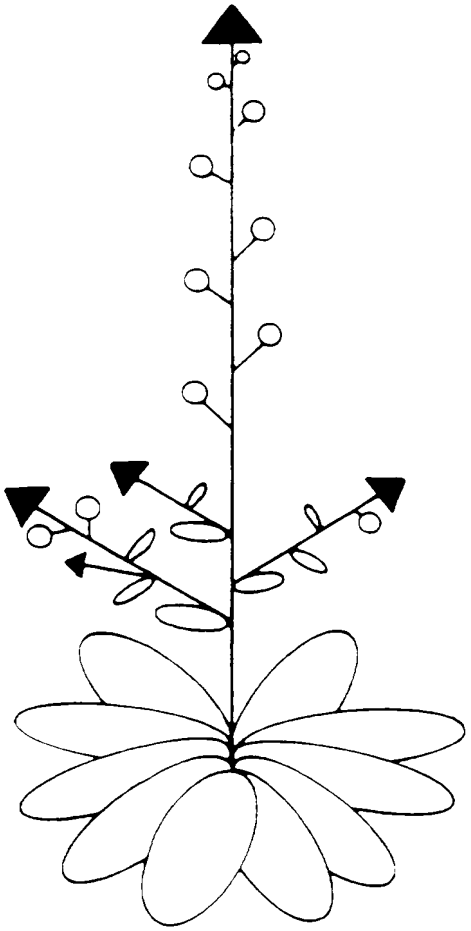


Fig.1 (b).

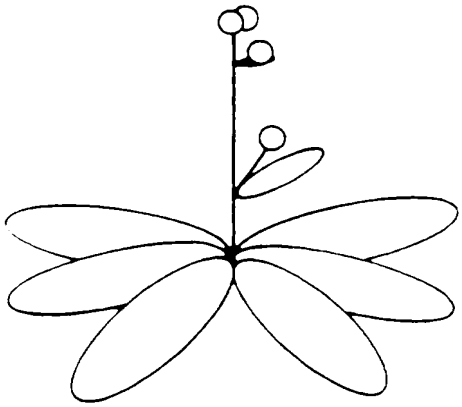
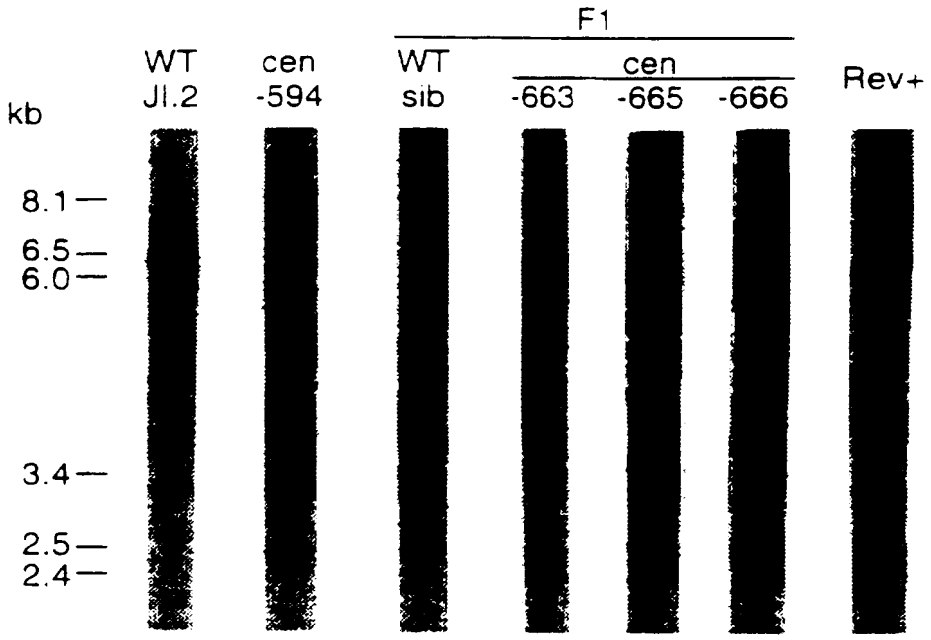


Fig.2.



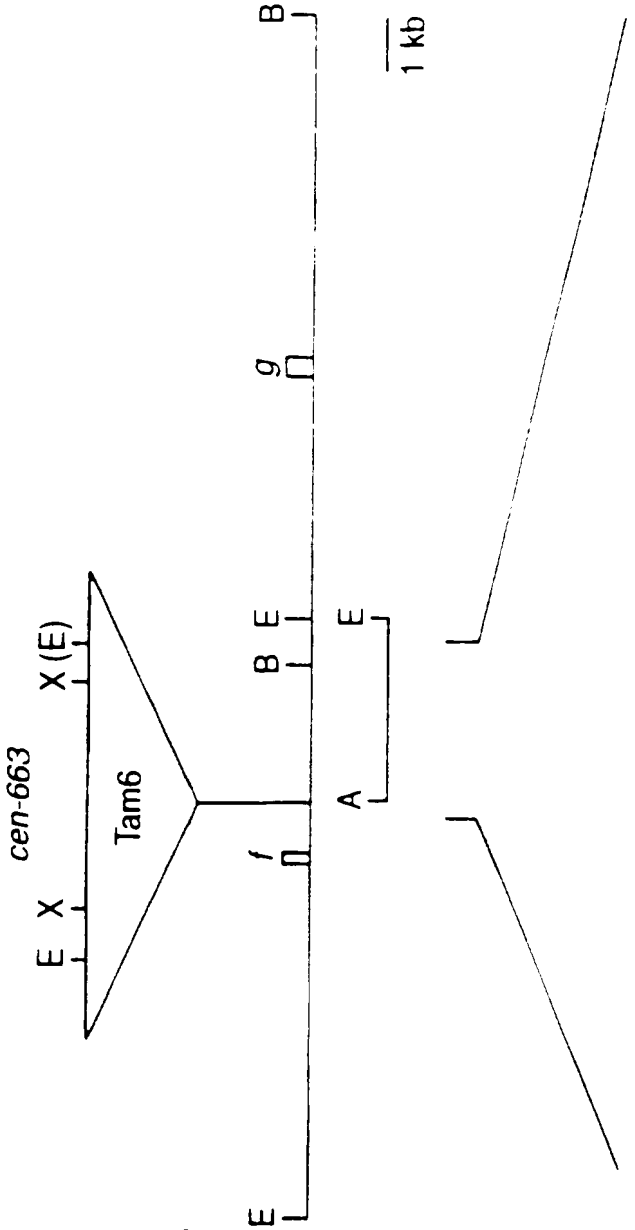


Fig.3 (a).

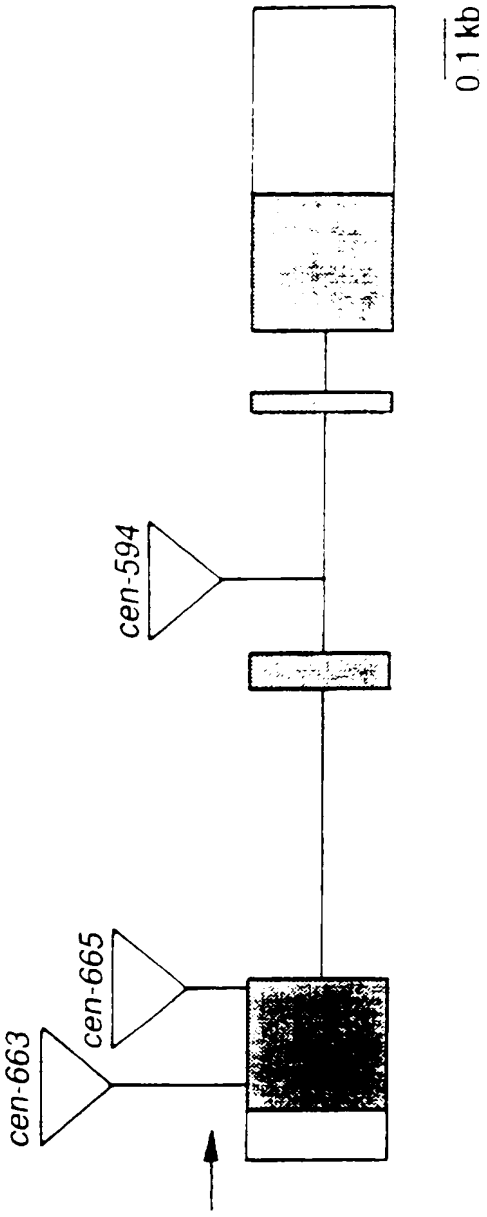


Fig.3 (b).

Figure 4(a)

```
   1  aagcaacatcaaaaacagcatcataaatccttttacttttgttgcatttttatcatctta   60

  61  attaagcattcttctccatataatatagttatggcagcaaaagtttcatcggacccgcta  120
  21                                  M  A  A  K  V  S  S  D  P  L    40

 121  gtgatagggagagttatcggagacgttgttgatcattttacctcaactgttaaaatgtct  180
  41  V  I  G  R  V  I  G  D  V  V  D  H  F  T  S  T  V  K  M  S    60

 181  gttatttacaactccaacaattccatcaagcatgtctacaatggccatgagctctttcct  240
  61  V  I  Y  N  S  N  N  S  I  K  H  V  Y  N  G  H  E  L  F  P    80

 241  tccgctgttacctctacacctagggttgaggttcatggtggtgatatgagatcatttttc  300
  81  S  A  V  T  S  T  P  R  V  E  V  H  G  G  D  M  R  S  F  F   100

 301  actctgataatgacagaccctgatgttcctggtcctagtgatccatacctgagggagcac  360
 101  T  L  I  M  T  D  P  D  V  P  G  P  S  D  P  Y  L  R  E  H   120

 361  ttgcactggatagtcacagatatcccagggaccactgattcctcattcggcaaagaagta  420
 121  L  H  W  I  V  T  D  I  P  G  T  T  D  S  S  F  G  K  E  V   140

 421  gtgagctatgagatgccaaggccgaacatagggatccacaggtttgtatttcttctgttc  480
 141  V  S  Y  E  M  P  R  P  N  I  G  I  H  R  F  V  F  L  L  F   160

 481  aaacagaagaaaagagggcaggcgatgttgagcccaccagtagtgtgcagggatggattc  540
 161  K  Q  K  K  R  G  Q  A  M  L  S  P  P  V  V  C  R  D  G  F   180

 541  aacacgagaaaattcacacaggaaaatgaattgggcctccctgttgccgctgtcttcttc  600
 181  N  T  R  K  F  T  Q  E  N  E  L  G  L  P  V  A  A  V  F  F   200

 601  aattgccagcgcgaaaccgctgccagaaggcgttgaacgtactatttatccatatcttat  660
 201  N  C  Q  R  E  T  A  A  R  R  R  *                          220

 661  ggctctgcatatatatatatatatatgctagtactactgatgtatcttcatcagggaaat  720

 721  aaatcatatgtagggtttcttttgcaatgataaagagtccctacgtctgctaccaaaaaa  780

 781  aattgttagagtggcctttgcaagtagtgaaaggatatgtgtacgtaatagggaaggaaa  840

 841  agatggagaaatgggaaattgtgatgtccacttgttataaattgatgtaattaatttcta  900

 901  tgatatataatttggaagttgtgttgtgc  929
```

Figure 4(b)

```
   1 TCATGTAACATGAAATCACTACCCTTACATGTGTCTTGGGCAGACGAAGTGGCCTCAATT   60
  61 CTAATTGAGCAAACATGGATAGGCAAACGAAACAAGACTTAGAAGACATTAAATCAATTT  120
 121 GCTCTAAGTAATGATCGAATTTGAGGTTAAAGGAGTGAATTACACTTCTTTAGCCAAATA  180
 181 TCAAATTGTACTTTTTAATACTCAATTTTTTATTCATTTGAAGACTGTGACTTCTTTAGT  240
 241 TCTTTTATTGTCTTCTTTCTCTTGTGATATATTACTTTTATTGAAAACGCTCGTTGAATA  300
 301 ATATGCAAAGCATATGATAAATTCATACCCCTCATATATCCGGTTGATTCTAATATTTTG  360
 361 CAAGAAGGACCACAACCCTTAGTTGGTTTTTCGTTTTCCTTTTGTTTCTGACTTCCACTG  420
 421 CCCTTGTTTCAAAATTTAATCACGACAAGAATTGGGACAGATAATTTGAATATTTCAATT  480
 481 CAGGGAAAAAAGGAAAATAAGAAATTACAGCTCGTTCTTTTAGAATGAATTAAAGTATTA  540
 541 AACAATTGGTACTTTGTTGAAAAACTACCACATCGTTACCGCTCTTATACCATTTAAACC  600
 601 CAAACCATTAATTGATTTTGGAACTTTTCAAAATTAATGATGTTTTAATTGCAACAAGTA  660
 661 ATTTGCTAGCATTTTAATCTATTTTATCTTCTATGTTACTTGTAGCAACACAACACCTTT  720
 721 TCGTGTGCTGTTATCAGATTTTGTATTCTCAATTATCGTATAAACCGTGAAGATATGCCC  780
 781 CTCGATCCACGGTCTTAAGCTTTCAATTATTTGAATATTGGAATCTTTGTCTCGGGTTTA  840
 841 TACCTGCAGCCAAGATATTCTCAATGTGCCATTCTTGGGTGCCATTTCATCCCTAATTAG  900
 901 AAATTACGAATTTTTTTTTTAAAATTTCTAGCACGGAAAGTTGTCTGTTTTGAAAAGACC  960
 961 AACTCGTGTATTTTATGCTATTGGCCAATTAGTTAATTTGTCATTTCCTTTTTTTTTTTG 1020
1021 TAATGTAAATTTTAGAATATGAAAGCACTAATGATTATGATGAAGTAAACACTTGTTAAT 1080
1081 TTTGATTCCTTTTCTTTTCTTTTAATATTTTCAGATATGTTTATAATTATTCATTAACAT 1140
1141 TTAATTATTCTTTTACTTTCTTTCCCACTTAAACATGAATTAAGAATGTTATTATGTTAT 1200
1201 TATGTAAAAAATTACAAACGTGCGCATTTTTATTTCTCTCTCTAAGCTCATGAATATATA 1260
```

Figure 4(b) Continued

1261 ATAATAATTTATTAACATTTAACAAATATATATCTGTAGAGATAAAGAAAAAAAAGTATT 1320

1321 ACCATCACACATATCATAGGAATATGCACCAGGATGGTGAGAAATAATAAGGTTGAAGTA 1380

1381 AAGAAAGATGACGAAAATGAAAAGAAAAAAAAAAAGAAAAAATTAAAAAAGGAGAAATTAT 1440

1441 ATGAGTTAGTTTGTTAATGCACCACTTATATAACCTTTAAAATAAATCATACCCCTTTTA 1500

1501 AAAGTGAATGTACAACACCCTTATGAATTGGATGAGGAGTTGTTCAAGTATGGGGCATTT 1560

1561 TATTTATAATATAATATAAAGGAGTTTCAATTGAATAATATCTAATGAAAAATATTGTTG 1620

1621 GGTGTAAATTTCTTGAACGATGATGGTGTATCTCATACTTTTTCACAAATATGTATGGTC 1680

1681 ACAGTTTATAATTATATATCTAAACATGTATATGTAAACTGAATATTGGCAAAAGTATAT 1740

1741 TGTACGGCCCAGGTATAAACTTATTATAGGGAAGATAAGCATTTGTTCTACTATATCACC 1800

1801 CCTTATTCGGTTAAGGCCCAACTTGATACTCCATTGGGCCTGAAGAGATTTCTTGAAAAG 1860

1861 CCTACTAACATTTGGGGCTTGAGGACGAGGTTCGAGTCCTGAATGGAGAATTTACATGAA 1920

1921 CCAGGATATGTAAGCGGTCCAAAAAGGCCCAAATTAATATAATTGATTTTATTATTACTA 1980

1981 AGTTCTATGCAGTAGTTGATTTGTTATCATTGTTTATCCACGTTATTAAGGATTACCTGA 2040

2041 GTTTATTTGTTTCCTACTTCTCATTCTAATCCTGAATTTTAGAAAAAATGATCCTACCTC 2100

2101 ACATATGTTAAGACTAAAATTTAATTTCTAGCAAAAGTTTCGATTTATTGGAACCAGAAA 2160

2161 GCTCTTTATGTCAATCAGCAATGAGCATAACTTTCTTCTCCATCCAATGATTCATAATTA 2220

2221 GATGATTAACAAATGATTAAGTGCAATATGAGTCACGAATCATCGAGTATTGTTCCTATT 2280

2281 ATTTAGTTATCAAATTAATCTAAGCATTTCCCCCGTCGAAGTTCAAATATGTCATATTAT 2340

2341 AAACGGAATTATGCCACCATACAATCTTAATATGTACGACGATTCTTTCGAGTTGCGACA 2400

2401 AATAGTTCTTAGCACTGACTTAAATTAAGGACCCTCTGAAGATATAGCAGAATATTACCG 2460

2461 TGTGTATATATATTATTCAATGACCAAAAGTGAAGCTCATTAAAATATAGAATTTAATTA 2520

Figure 4(b) Continued

2521 CCGTGTATATATATATATATATATATATATATATATATATATATATATATAACCACATTC 2580

2581 ATATTACGTATAACTTGTAAATCAAAGGTTGGCTTAATAGTGTAAGATCCTATTGAGTTC 2640

2641 TCACGGGTGGATGCGATCTATTTAGCAAAACGTCACGAATTTGATCCCTAGCATGTGCAA 2700

2701 ATTTCATTGCGTCAGTACAACCATGATTCGTGAGCAAAAAATTGTTATTTTCGGGGTGCA 2760

2761 CTTTAAAAAATTCGGGCAGAGTGTTGAGACATAAATTGAACTTTTTGTCTTTAAAACGATA 2820

2821 TTGCCCCGTTACGGTGCTAACCTAATACTATATTTTAAGTAATCGTTTCATAAGTATACA 2880

2881 CGTATAAGTAAAAATAATAGCAAAATGAGCGTATTGAGCTCACCGTTTTTGAATAAAATA 2940

2941 ACAAATTTACATCGGATGAGAACCGCATCGCCGCAGGAAAAAAGAAGGGTGAAGGAGAGA 3000

3001 GATACAAATAAGAAGAAGCAAAAGCTTGAGTATAGATACTCAAGGTATAGAAGTCAAGTT 3060

3061 CAACTAGAGCAAACTATTAAGAAATTAAATAAAGCATTAGGACTTACTTCTTATAGCAAA 3120

3121 CGAACCCTCCCCCACCTTGCTACATTAGGGATAGCTAAAACTCAAAATTTATTCCCTTCT 3180

3181 TTTCGTTGAGATGACCTCTCAACTCATTGTAAAATGACATGCCATCAATTGTGGAGTTCC 3240

3241 TTTTATGTATGCGCTGATGAAACCTTCTTTATTTATTCTCCTCATATACACACAAATGTC 3300

3301 ATGCTGGAGAACCTTAGAACCTCCACTTTTATTCCTTAAATACAAAAGCTCATAACTCTT 3360

3361 TTGGTAGCTGCAAATGTGCAAACAGTATCCAGAAATTCTATTTGCCCTTTCTTTACATTA 3420

3421 AAAAAGGAATTACAAAGATGAACATCCTCACCCTATAGAAATTAATGGGGTAATAGCAAA 3480

3481 AAGTACTCGATGTTATTTCTAATTGGCAAAAGAATCACTGTGTTATTTTAATTAGCAAAA 3540

3541 GAACCTTGTCTTATTCGGTAAATGGCAAGAAAAAAATTGGCTTCTAGTTTGGAACTACAC 3600

3601 ATGGTCAATGTGAGTCTTTGCTCCTGACTTACAACCATTTTTGATGATTTTCCCCACTCT 3660

3661 TCCGTAATGCTTCAGTGTTTTAATAAAATTAGCAAAAAACATCCCCTTGTGTTTTTATGA 3720

3721 AATTGGCAATAACCTCCCTGTGTTTCATATAATTGGCAATAACCCCCTCTTCTATATACG 3780

Figure 4(b) Continued

```
3781  TTTCCTTCAATCAGATGTATCAATTTCACGGGGTTCGAGGAAGTAAGCTTAAAAAGCATA  3840

3841  ATTTTACCTGCTATTAACGCCCAAAAACAAAATGAGAATATGCTAATTATCGAAAAACAC  3900

3901  ATGCATGTTCTTTTTTTGCCAATCAAAATGACATTGGGGGTTTATTGTCAATTAAAAATA  3960

3961  ACACGAGGCTAGTTTTTGTTAATAGCTCAGAAATCAATACCTAATTAACCACGCAGTATT  4020

4021  AATTTTACATTTTATGTGAGTGTCAGAGAGATATAAGAGATACATAAGCGTGGCATGTCA  4080

4081  AAATCATCTTTAATAAGTATACTTCTTGCTTTTGTATATTTTTTTTTTCCAAAAGAAAAA  4140

4141  ACATTCGTCGTAGCTTGGTTGCCTGCCAGATAATGTCTAAAACCAATGTGTCATAGCTAG  4200

4201  ATGGCTGGGTTTTACCCACTTTGAAACTCCCTTAATTCAGTATTTTAATCAAAATTCTCC  4260

4261  TCGCACTGCAATGATCTGCGAGTTGCTTGTAGCCACTATAAATATATGGGGTTTGCTATT  4320

4321  CCATTCTaagcaacatcaaaaacagcatcataaatccttttacttttgttgcatttttat  4380

4381  catcttaattaagcattcttctccatataatatagttatggcagcaaaagtttcatcgga  4440
1461                                          M  A  A  K  V  S  S  D  1480

4441  cccgctagtgatagggagagttatcggagacgttgttgatcattttacctcaactgttaa  4500
1481   P  L  V  I  G  R  V  I  G  D  V  V  D  H  F  T  S  T  V  K  1500

4501  aatgtctgttatttacaactccaacaattccatcaagcatgtctacaatggccatgagct  4560
1501   M  S  V  I  Y  N  S  N  N  S  I  K  H  V  Y  N  G  H  E  L  1520

4561  ctttccttccgctgttacctctacacctagggttgaggttcatggtggtgatatgagatc  4620
1521   F  P  S  A  V  T  S  T  P  R  V  E  V  H  G  G  D  M  R  S  1540

4621  atttttcactctgGTATTGTTTTACTATTCTGTGCTACTTATCTCTTAGGTTAATTATTG  4680
1541   F  F  T  L                                                  1560

4681  TGAACTCTCTATACCCTAAAATGAAAGATATTTTTGAACCTTCAATGTAATAAGTTCTAC  4740

4741  ATGTGAGGTTCCTATCAAAATTTATCTATCAAAATTGTGCAATACTTTTTGTAGTGTTAC  4800

4801  TAGATATATGTCATGTGTAAATATGATAAATACAAGATAAAAACTTAGATACTTTTTTCT  4860

4861  CTATCCACCCATCACTGCATGCATGGATTAAGGTCACGCCATACATTATATACACATGTC  4920

4921  GTTACTCTAATAGCGATATATAGAGTGGTAACGATTTTTTGGTACAGAAATGGTGCTGTA  4980
```

Figure 4(b) Continued

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4981  AGTTATACAGATGTTCACAACCACTTAAACTTTTCGTAGTTTTGAGGAATGTTATTTAGT  5040

5041  GTGTAGAATATTTAATATCTTGAAGCAATTAATTTTGAGAGATTTACTCAATTAGTTTGT  5100

5101  TTGTTTCAGataatgacagaccctgatgttcctggtcctagtgatccatacctgagggag  5160
1701            I  M  T  D  P  D  V  P  G  P  S  D  P  Y  L  R  E    1720

5161  cacttgcactgGTAAATATGCTTACTTTGGAACTTTCTTCACACACTAGAAAAATAACAC  5220
1721  H  L  H  W                                                    1740

5221  AAAAGATCATCAAGCCCTAAATTTTTCCTTGCATGGAGGAACATATATAACAGGGATTCT  5280

5281  TTCACATTGAGTAAACAAAAGTCACTAGCGAAATGTATAGCTAACCAGTTTATGACAATT  5340

5341  CAAGCTGTTTTAATCATTCTTCCAATTAATGGCCATATATATATATATATATATACTCCC  5400

5401  GATAAAAAAATGAATCTTTTCAAGAAAATTTTGTCAGCTGCAATGATTCAATCAGCTTTCT  5460

5461  TGAAAATCCCATAAAAGAAATGAACAACTTGCTAATTATGCATTTGATACTTAAAGAGTA  5520

5521  CAAGTTTAATTATGTCACCCCGCTGATATAACTTGATTTGACTAACTCGCAGgatagtca  5580
1841                                                    I  V  T    1860

5581  cagatatcccagggaccactgattcctcattcgGTATGATTAAATTTTCCCTCCACATTT  5640
1861    D  I  P  G  T  T  D  S  S  F  G                            1880

5641  AAACCAAAATACATTAATAATAATACCCAAATAAATATTCCACCATGACTAATTAATTAA  5700

5701  TAAATTGTTGCAGgcaaagaagtagtgagctatgagatgccaaggccgaacatagggatc  5760
1901                  K  E  V  V  S  Y  E  M  P  R  P  N  I  G  I    1920

5761  cacaggtttgtatttcttctgttcaaacagaagaaaagagggcaggcgatgttgagccca  5820
1921  H  R  F  V  F  L  L  F  K  Q  K  K  R  G  Q  A  M  L  S  P    1940

5821  ccagtagtgtgcagggatggattcaacacgagaaaattcacacaggaaaatgaattgggc  5880
1941  P  V  V  C  R  D  G  F  N  T  R  K  F  T  Q  E  N  E  L  G    1960

5881  ctccctgttgccgctgtcttcttcaattgccagcgcgaaaccgctgccagaaggcgttga  5940
1961  L  P  V  A  A  V  F  F  N  C  Q  R  E  T  A  A  R  R  R  *    1980

5941  acgtactatttatccatatcttatggctctgcatatatatatatatatgctagtacta    6000

6001  ctgatgtatcttcatcagggaaataaatcatatgtagggtttcttttgcaatgataaaga  6060

6061  gtccctacgtctgctaccaaaaaaaattgttagagtggcctttgcaagtagtgaaaggat  6120
```

Figure 4(b) Continued

```
6121  atgtgtacgtaatagggaaggaaaagatggagaaatgggaaattgtgatgtccacttgtt  6180
6181  ataaattgatgtaattaatttctatgatatataatttggaagttgtgttgtgcAAATTTT  6240
6241  GAAGGGCTTAATTTTTGAATGGTTGCAAAAATTATTCTTTATCTTTTCTTTTTAAAACGT  6300
6301  GGAAGCACAATCATTAATGTCTCTTTGTTTGGTAAACATTTATGTGTATGTCTACAATTT  6360
6361  TTATCGTTTATTTGTACTAATAATTTTAGTTTCGAACATGCAATGTTTGACCTTTTCCTA  6420
6421  TCCGATTGATCATGTGGTTTTTTGATATTATTCTTTGAAGAGTGCTTATGCTTGTCAGGG  6480
6481  CGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGG  6527
```

Figure 5

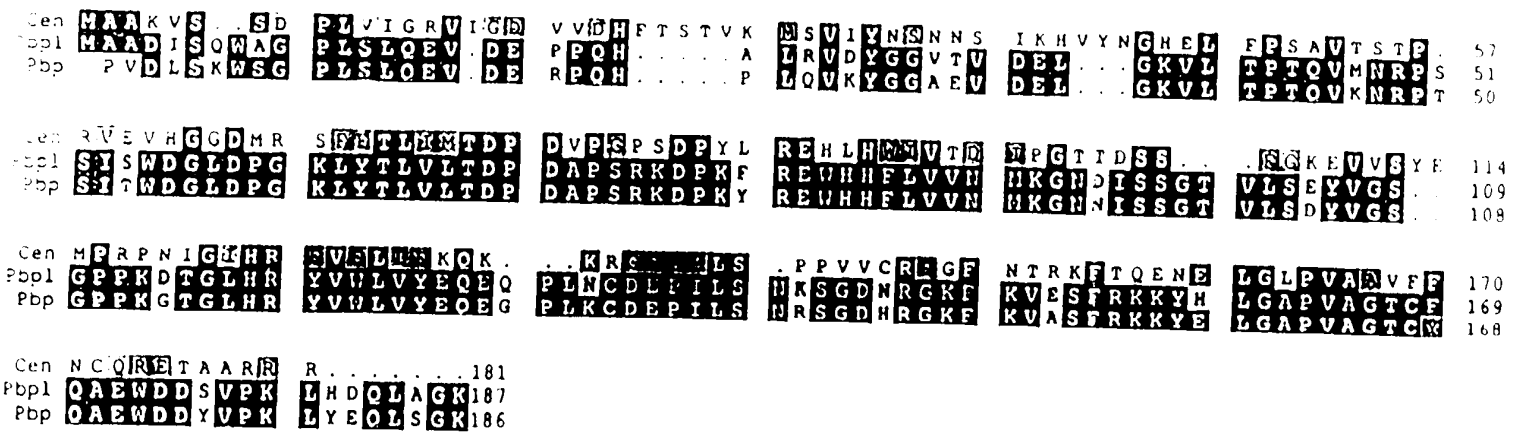


Figure 6(a)

```
     agttaacaaaagaaa atg gag aat atg gga act aga gtg ata gag 45
                     Met Glu Asn Met Gly Thr Arg Val Ile Glu 10

cca ttg ata atg ggg aga gtg gta gga gat gtt ctt gat ttc ttc  90
Pro Leu Ile Met Gly Arg Val Val Gly Asp Val Leu Asp Phe Phe  25

act cca aca act aag atg aat gtt agt tat aac aag aag caa gtc  135
Thr Pro Thr Thr Lys Met Asn Val Ser Tyr Asn Lys Lys Gln Val  40

tcc aat ggc cat gag ctc ttt cct tct tct gtt tcc tcc aag cct  180
Ser Asn Gly His Glu Leu Phe Pro Ser Ser Val Ser Ser Lys Pro  55

                                                (tfl1-14) t
agg gtt gag atc cat ggt ggt gat ctc aga tcc ttc ttc act ttg  225
Arg Val Glu Ile His Gly Gly Asp Leu Arg Ser Phe Phe Thr Leu  70

gtg atg ata gac cca gat gtt cca ggt cct agt gac ccc ttt cta  270
Val Met Ile Asp Pro Asp Val Pro Gly Pro Ser Asp Pro Phe Leu  85

    a (tfl1-13)                                   a (tfl1-11)
aaa gaa cac ctg cac tgg atc gtt aca aac att ccc ggc aca aca  315
Lys Glu His Leu His Trp Ile Val Thr Asn Ile Pro Gly Thr Thr 100

                  a (tfl1-1)
gat gct acg ttt ggc aaa gag gtg gtg agc tat gaa ttg cca agg  360
Asp Ala Thr Phe Gly Lys Glu Val Val Ser Tyr Glu Leu Pro Arg 115

cca agc ata ggg ata cat agg ttt gtg ttt gtt ctg ttc agg cag  405
Pro Ser Ile Gly Ile His Arg Phe Val Phe Val Leu Phe Arg Gln 130

aag caa aga cgt gtt atc ttt cct aat atc cct tcg aga gat cac  450
Lys Gln Arg Arg Val Ile Phe Pro Asn Ile Pro Ser Arg Asp His 145

ttc aac act cgt aaa ttt gcg gtc gag tat gat ctt ggt ctc cct  495
Phe Asn Thr Arg Lys Phe Ala Val Glu Tyr Asp Leu Gly Leu Pro 160

gtc gcg gcc gtc ttc ttt aac gca caa aga gaa acc gct gca cgc  540
Val Ala Ala Val Phe Phe Asn Ala Gln Arg Glu Thr Ala Ala Arg 175

aaa cgc tag ttt cat gat tgt cat aaa ctg caa aaa tga aag aag  585
Lys Arg  *                                                  177

aaaatttgcatgtaatctcatgtttatttgtgttctgaatttccgtactctgaataaaa  644
actgccaaagatgagttgaatccg                                     668
```

Figure 6(b)

```
   1  cttagtattagaaataatgtagttagtgtacgtacatgtttgagacagcaaaaaaaataa   60

  61  aagaaagaaagaaaaaaggttagtacacataattgggaattaatgtctattgattctttt  120

 121  atctttctctctctctctctaagatggaaaacccctataaatagatgtctcggtcgtctc  180

 181  tttgtctcccacatcactacaaatctctctttttcctctaagttaacaaaagaaaatggag  240
  61                                                      M  E       80

 241  aatatgggaactagagtgatagagccattgataatggggagagtggtaggagatgttctt  300
  81  N  M  G  T  R  V  I  E  P  L  I  M  G  R  V  V  G  D  V  L   100

 301  gatttcttcactccaacaactaagatgaatgttagttataacaagaagcaagtctccaat  360
 101  D  F  F  T  P  T  T  K  M  N  V  S  Y  N  K  K  Q  V  S  N   120

 361  ggccatgagctctttccttcttctgtttcctccaagcctagggttgagatccatggtggt  420
 121  G  H  E  L  F  P  S  S  V  S  S  K  P  R  V  E  I  H  G  G   140

 421  gatctcagatccttcttcactttgGTAAATAAATATATTTAAATTATTTTATAATAATGT  480
 141  D  L  R  S  F  F  T  L                                       160

 481  TGGTTTTATTTATATTGTGCCAAAAAAAACCATATAAAaCGTCTCACTTCCTTTTCCTCT  540

 541  TACAAGTTTTCCATTTCTAACTCAATAATCTTATAAACTTGTAGCTTTAGTTTTTATCAT  600

 601  TCCTTTTTCCAGTCTTTTTTTTTTAATGGTAAAACTCAACCGAAATGCAAAACAGgtgat  660
 201                                                      V  M       220

 661  gatagacccagatgttccaggtcctagtgacccctttctaaaagaacacctgcactgGTA  720
 221   I  D  P  D  V  P  G  P  S  D  P  F  L  K  E  H  L  H  W      240

 721  CGTTTAATTTATTTATTCTTTCTTTTCATTTTGGGCCCATATTCCATATACATTGCATTT  780

 781  AAATCATTTCGTTATAACCCTAATAAAGTTTTTTTTTGGGTGTAAGTTATATACATTTGAG  840

 841  TTGGTCAAAGATCTCCATCGCCATGAGTTCTCAGAACTTTTTCTGTAAAGTAATAATATT  900

 901  AGTATTGTTGAATGTTTCAATAGgatcgttacaaacattcccggcacaacagatgctacg  960
 301                              I  V  T  N  I  P  G  T  T  D  A  T  320

 961  tttgGTAAGGCCTCTTCATGAATCTTGTAATTTAAATACTTATACATATATCATGTTATA 1020
 321  F  G                                                         340

1021  TAGAAATAAAAATATTTGCATTGTAATATAGgcaaagaggtggtgagctatgaattgcca 1080
```

Figure 6(b) Continued

```
341                                                                  K  E  V  V  S  Y  E  L  P      360

1081  aggccaagcatagggatacataggtttgtgtttgttctgttcaggcagaagcaaagacgt                                1140
 361  R  P  S  I  G  I  H  R  F  V  F  V  L  F  R  Q  K  Q  R  R                                 380

1141  gttatctttcctaatatcccttcgagagatcacttcaacactcgtaaatttgcggtcgag                                1200
 381  V  I  F  P  N  I  P  S  R  D  H  F  N  T  R  K  F  A  V  E                                 400

1201  tatgatcttggtctccctgtcgcggccgtcttctttaacgcacaaagagaaaccgctgca                                1260
 401  Y  D  L  G  L  P  V  A  A  V  F  F  N  A  Q  R  E  T  A  A                                 420

1261  cgcaaacgctagtttcatgattgtcataaactgcaaaaatgaaagaagaaaatttgcatg                                1320
 421  R  K  R  *                                                                                  440

1321  taatctcatgtttatttgtgttctgaatttccgtactctgaataaaaactgccaaagatg                                1380

1381  agttgaatccgAAATATCAATTGAGTTTACAGAAGTATTGATAACGATCT                                          1430
```

Figure 7(a)

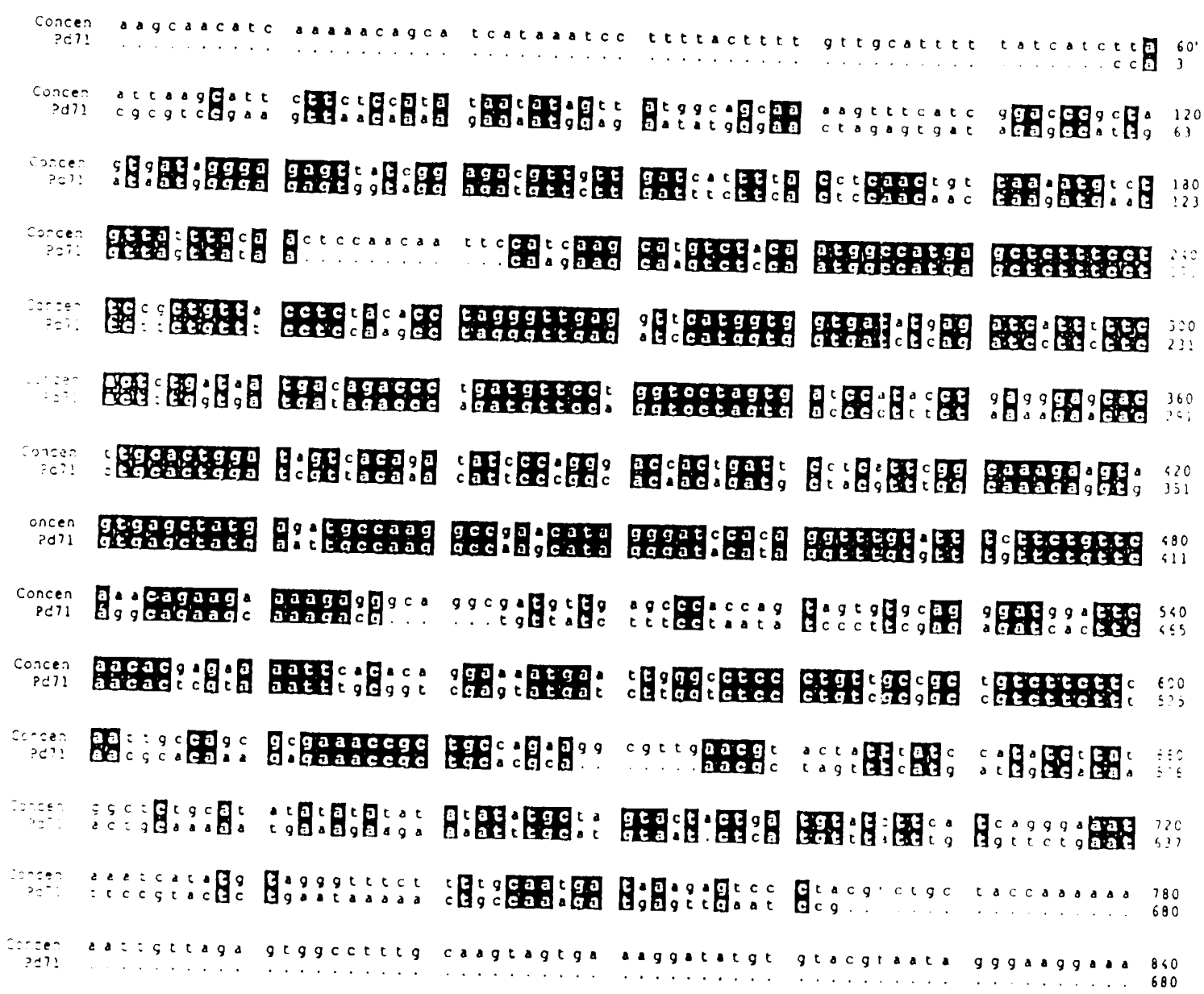


Figure 7(a) Continued

```
Concen  agatggagaa atgggaaatt gtgatgtcca cttgttataa attgatgtaa ttaatttcta 900
Pd71    .......... .......... .......... .......... .......... .......... 680

Concen  tgatataaa  ttgggaagtt gtgttgtgc929
Pd71    .......... .......... .......680
```

Figure 7(b)

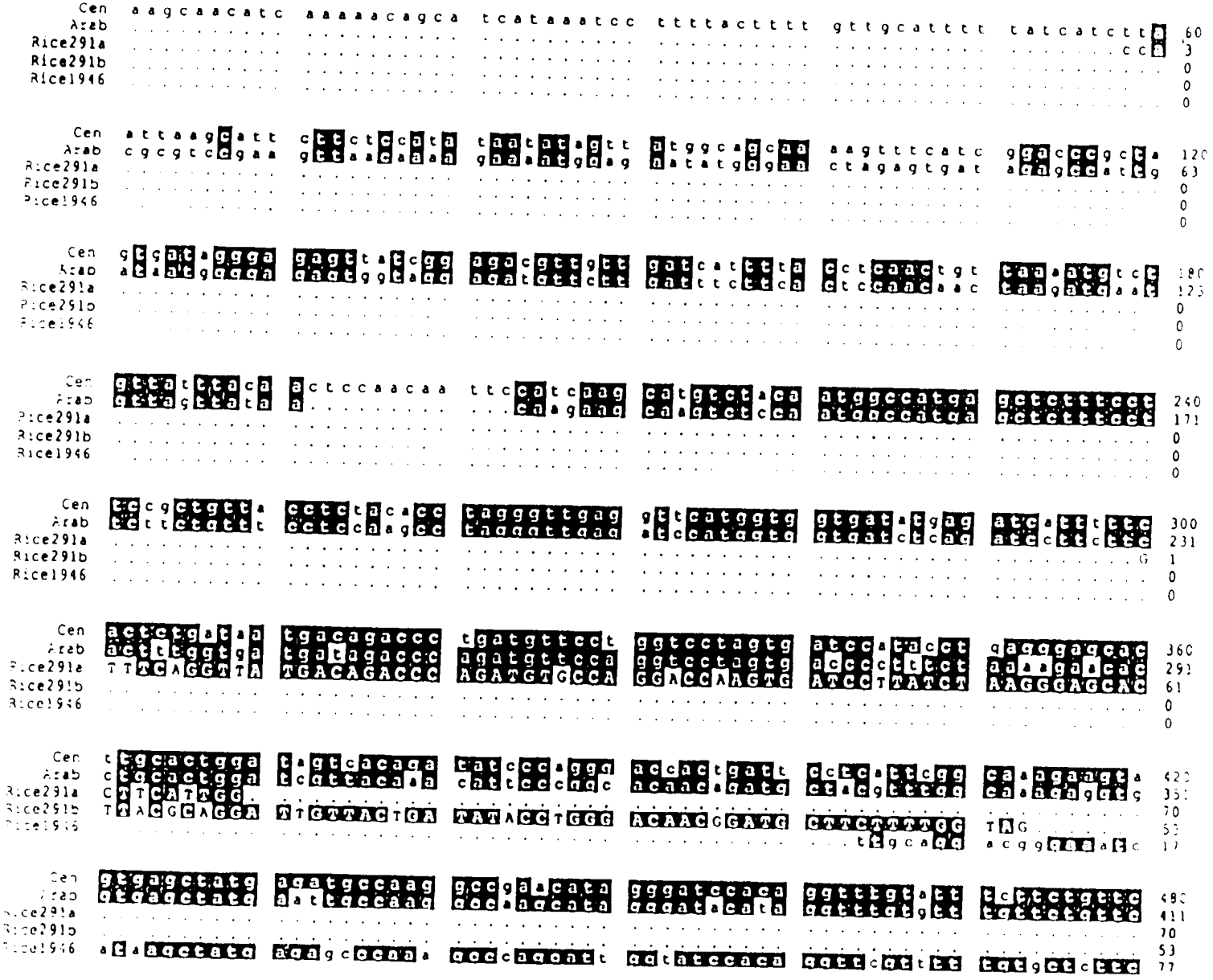


Figure 7(b) Continued

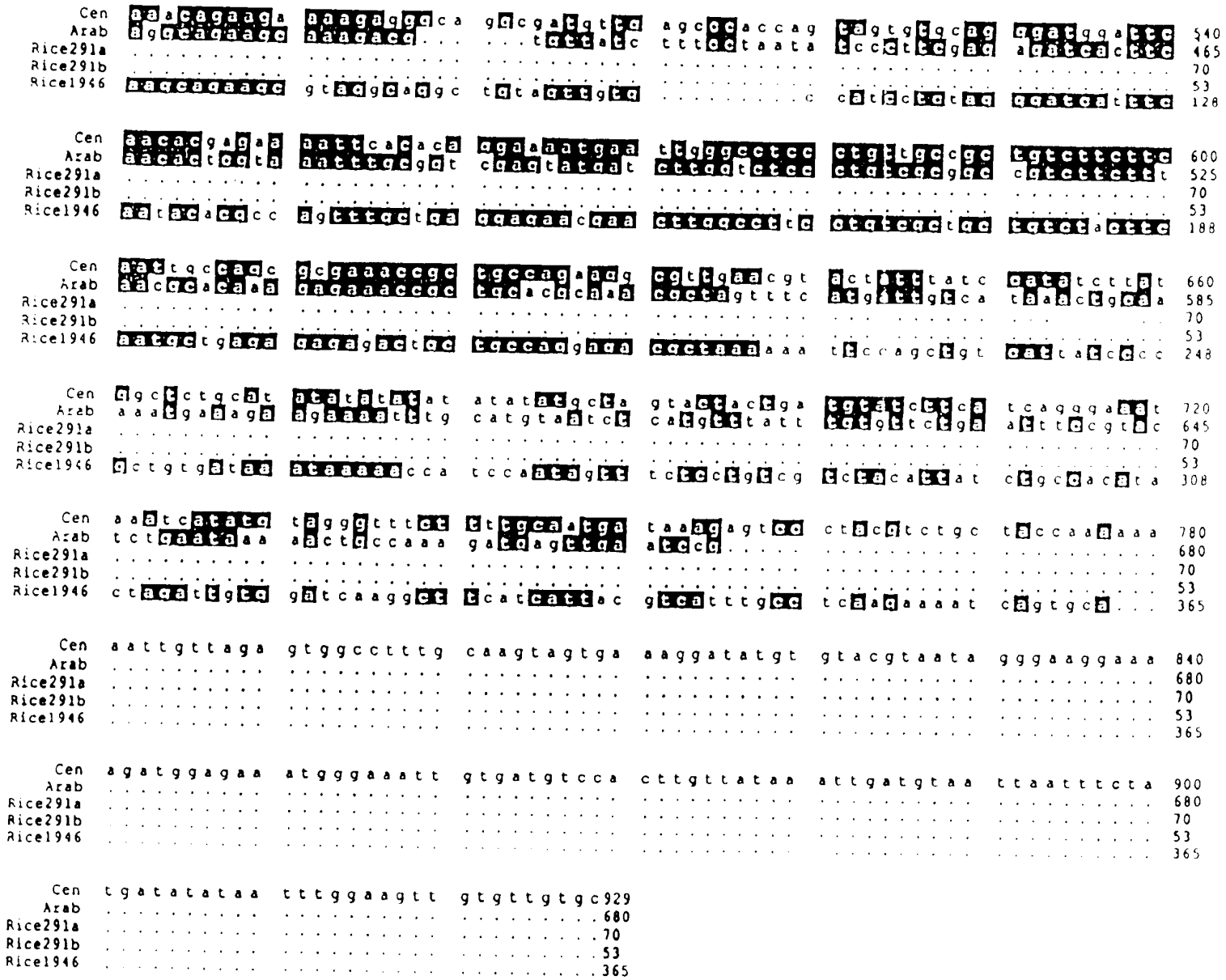
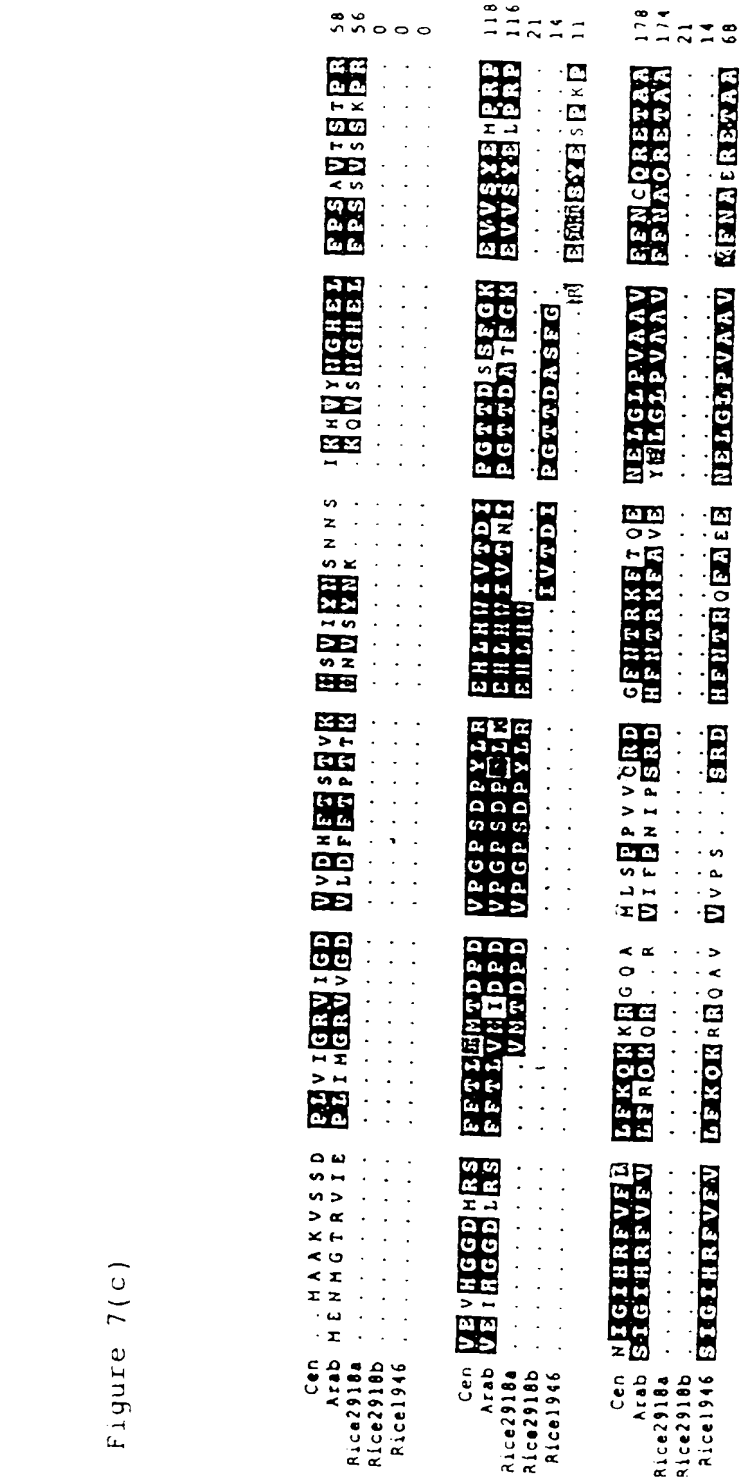


Figure 7(c)



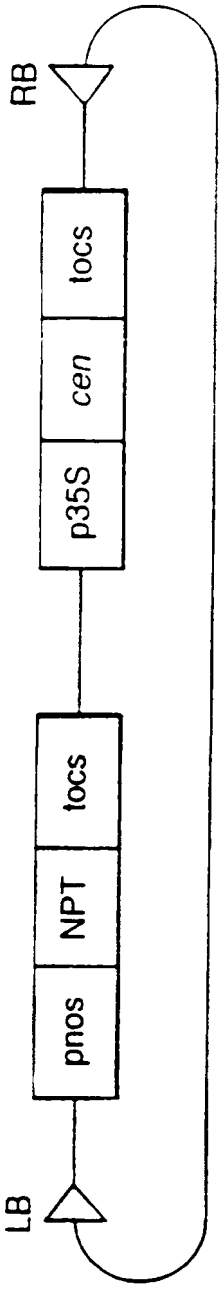

RB
tocs
cen
p35S
tocs
NPT
pnos
LB

Fig.8 (a).

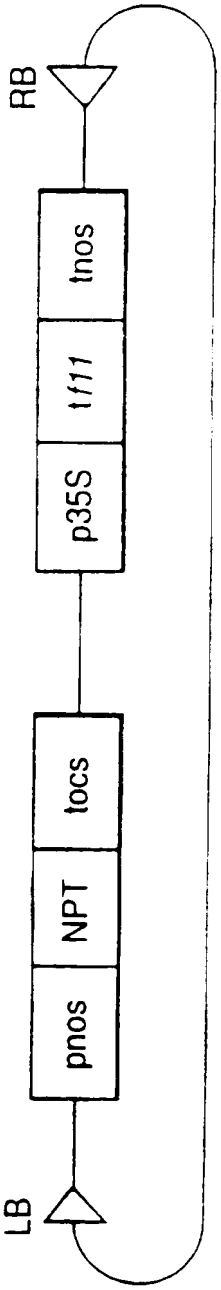

RB
tnos
tf11
p35S
tocs
NPT
pnos
LB

Fig.8 (b).

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/02276

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/82 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL SEQUENCE DATABASE. REL. 42. 4-FEB-1995. ACCESSION NO. T44654, XP002024908 NEWMAN, T., ET AL.: "7917 Arabidopsis thaliana cDNA clone 129D7T7" see sequence ---	3-6,8,9, 11,14, 15,22,23
P,X	NATURE (LONDON) 379 (6568). 1996. 791-797. , XP002024909 BRADLEY D ET AL: "Control of inflorescence architecture in Antirrhinum." see the whole document --- -/--	1-10,18, 22,23

[X] Further documents are listed in the continuation of box C.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 February 1997	20.02.97

Name and mailing address of the ISA	Authorized officer
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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DEVELOPMENT (CAMBRIDGE) 0 (SUPPL.). 1994. 107-116., XP000617343 COEN E S ET AL: "Evolution of flowers and inflorescences." see the whole document	1-35
A	THE PLANT CELL, vol. 3, 1991, pages 877-892, XP002024910 SHANNON, S., ET AL.: "A mutation in the Arabisdopsis TFL1 gene affects inflorescence meristem development" see the whole document	11-15
A	THE PLANT CELL, vol. 5, no. 6, June 1993, pages 639-655, XP002024911 SHANNON, S., ET AL.: "Genetic interactions that regulate inflorescence development in Arabidopsis" see the whole document	11-15
A	TRENDS IN BIOTECHNOLOGY, vol. 13, no. 9, September 1995, pages 350-355, XP002024912 MOL, J.N.M., ET AL.: "Floriculture: genetic engineering of commercial traits" see page 353, left-hand column, paragraph 2 - page 354, left-hand column, line 1	1-35
A	PLANT MOLECULAR BIOLOGY, vol. 25, 1994, pages 335-337, XP002024913 AN, G., ET AL.: "Regulatory genes controlling flowering time or floral organ development" see the whole document	1-35
A	TRENDS IN BIOTECHNOLOGY, vol. 9, January 1991, pages 31-37, XP002019122 BALCELLS, L., ET AL.: "Transposons as tools for the isolation of plant genes" see page 34 - page 35	1-15
A	CELL, vol. 80, 24 March 1995, pages 847-857, XP002004926 PUTTERILL J ET AL: "THE CONSTANS GENE OF ARABIDOPSIS PROMOTES FLOWERING AND ENCODES A PROTEIN SHOWING SIMILARITIES TO ZINC FINGER TRANSCRIPTION FACTORS"	30-34

-/--

2

(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THE PLANT CELL, vol. 3, 1991, pages 359-370, XP002024914 MEDFORD, J.I., ET AL.: "Molecular cloning and characterization of genes expressed in shoot apical meristems" see the whole document -----	35

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